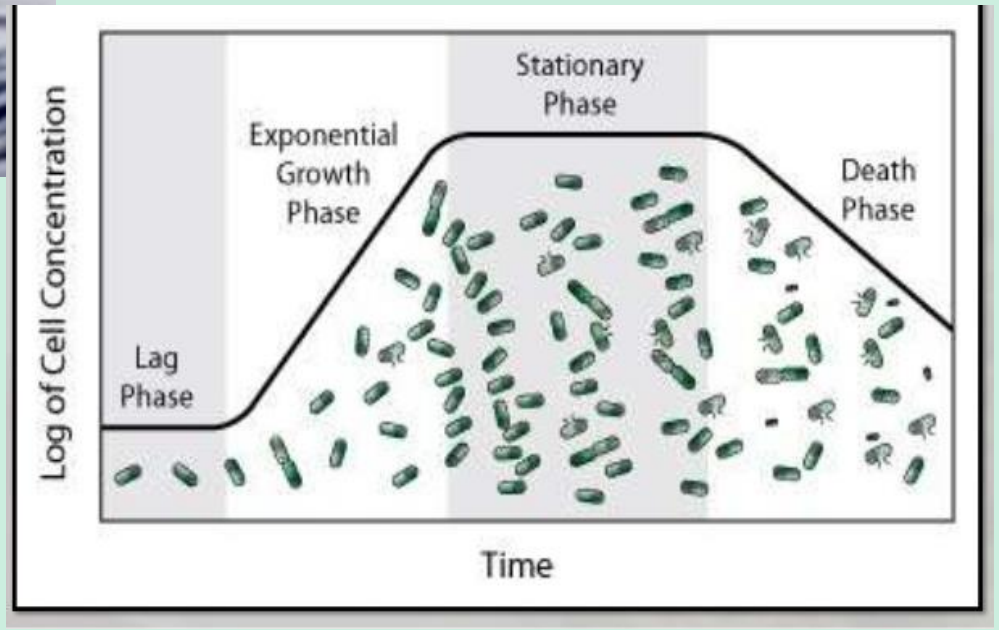
Chapter 6 Microbial Growth



Chapter 6 Objectives #1-5

1. Classify microbes into 5 groups on the basis of preferred temperature ranges and
   A. Special characteristics: animal pathogen, refrigerator spoilage, “extreme” locations...
2. Identify optimal pH of most bacteria.
3. Explain the importance of osmotic pressure to microbial growth and/or lysis, and food preservation.
   A. What direction does water flow in hypertonic vs. hypotonic environments?
   B. Does cell wall protect? Plasmolysis or osmotic lysis likely to happen?
4. Provide a use for each of the CHONPS needed in large amounts for microbial growth.
5. Identify ways in which aerobes avoid damage by toxic forms of oxygen such as superoxide free radicals and peroxide anion.
   A. Know the enzymes (SOD & catalase) and their reactions.

Chapter 6 Objectives #6-8

6. Classification based on oxygen requirements for the following groups: aerobes, obligate anaerobes, aerotolerant anaerobes, microaerophiles, and facultative anaerobes.
   A. Identify optimal incubation conditions and relative growth rates in other conditions they will tolerate.
      i. Growth aerobic, candle jar, anaerobic conditions.
      ii. Growth locations and amounts in thioglycollate.
   B. Relative energy production between groups and in differing conditions (aerobic vs. anaerobic)
   C. Which groups based have SOD and/or catalase.
7. Describe and explain various means AND their mechanisms to grow anaerobes: reducing media, thioglycollate, anaerobe jars and their pouches, anaerobic incubators/hoods.
8. Media:
   A. How & why the pH of culture media is controlled.
   B. Distinguish between chemically defined and complex media
   C. Distinguish between differential, selective and general nutrient media

Objectives Continued

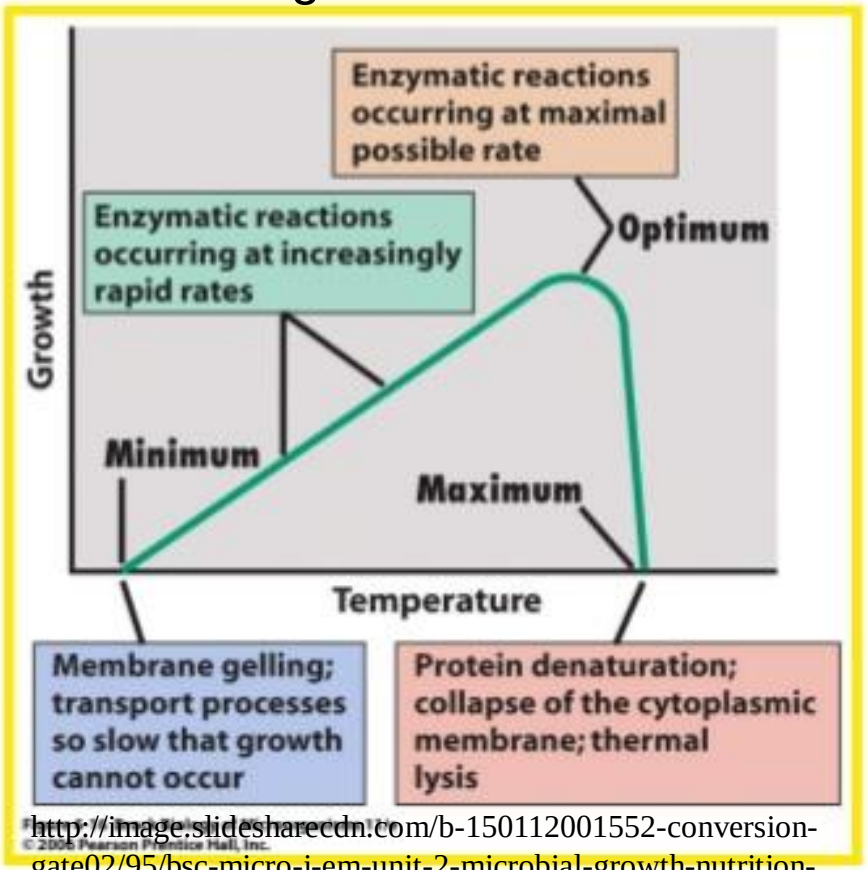
9. For each of the following: PEA, O-F glucose, starch, EMB, TSI
   A. Is it selective? If so, what does it select for? What does it inhibit? EXPLAIN its inhibition mechanism.
   B. Is it differential? What specific ingredient does the media contain to differentiate? What are the 2 (or more) groups and what is their appearance? Explain mechanism that causes diff in appearance.
   C. Interpret plates, tubes or descriptions of growth.
   D. For TSI –interpret glucose, sucrose/lactose and peptones ?H2S?
10. Explain methods to preserve microbes.
11. Bacterial growth:
   A. Define generation time and use it to calculate organism numbers.
   B. Compare the phases of a microbial growth curve.
12. Metabolism: Compare/contrast anabolism vs. catabolism, oxidative vs. fermentative, dehydration synthesis vs. hydrolysis
13. Define/explain pure culture, binary fission, turbid, aseptic technique, amylase, halophile, acidophile, exoenzyme vs. endoenzyme

Requirements for Growth

1. Physical = Environment/Surroundings
2. Chemical = Nutrients to Take In

Physical Requirements

1. Temp
   - A. Minimum Growth Temp: Lowest will GROW in
   - B. Maximum Growth Temp: Highest that will still grow in
   - C. Optimum: Grows best/fastest



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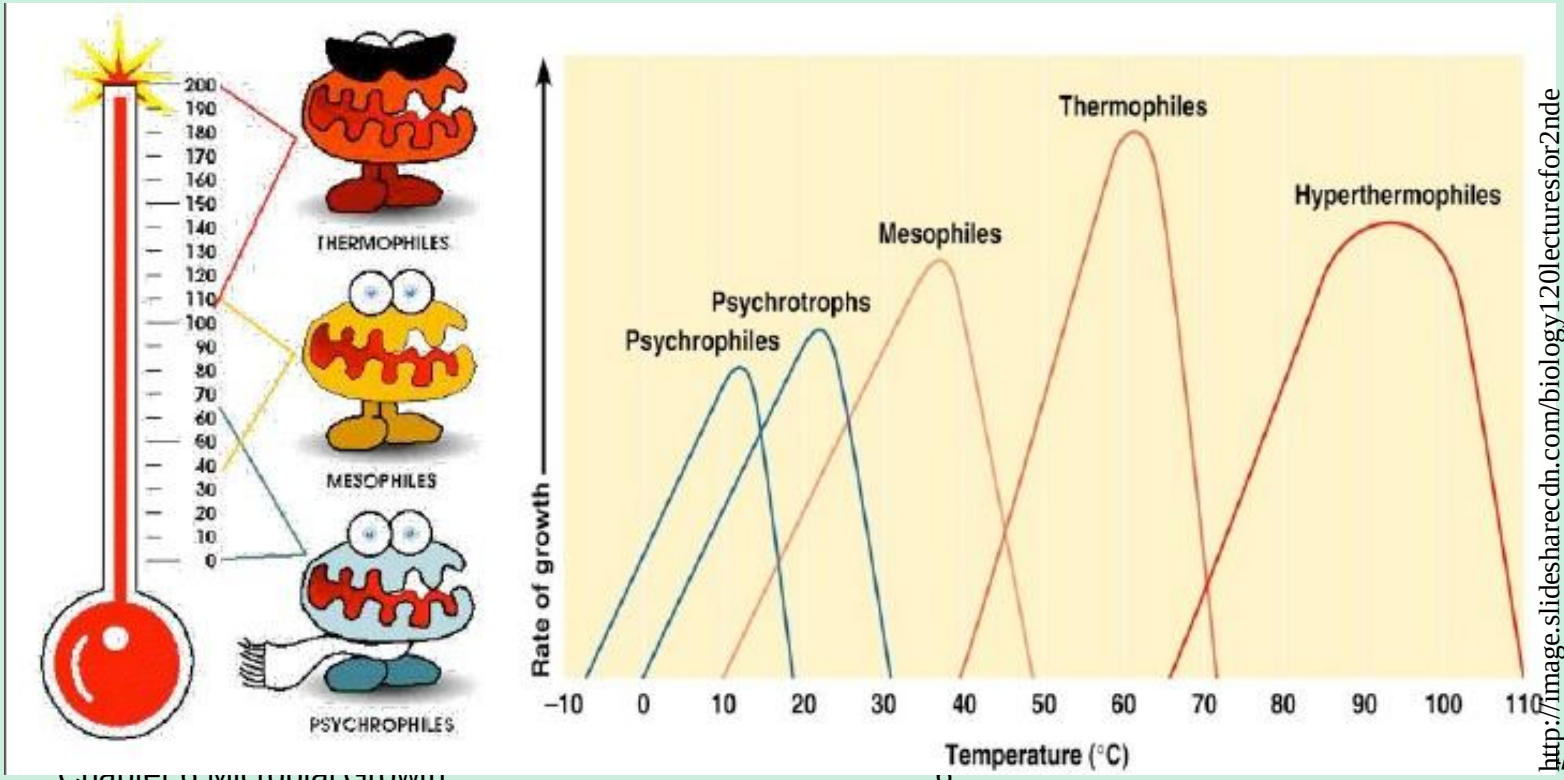
Temperature Growth Classifications

1. Psychrophile (cold-loving):
   - » Grows at 0°C
   - » Optimum 15°C
2. Psychrotroph:
   - » Grows at 0°C
   - » Optimum 25°C
   - » Refrigerator spoilage



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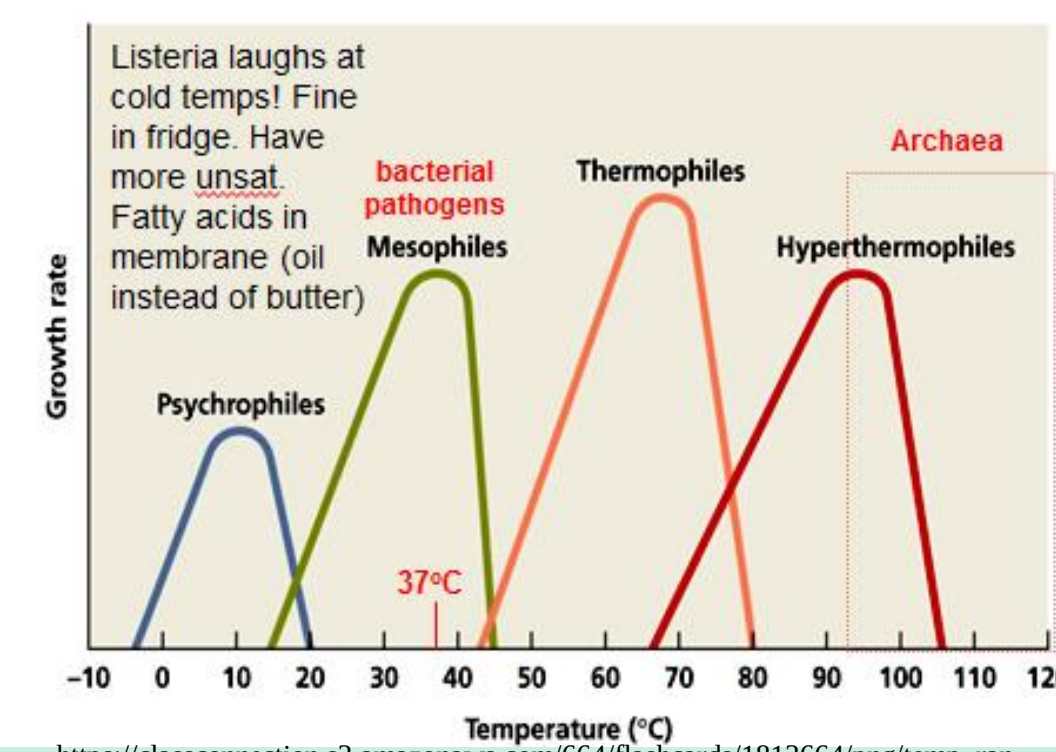
part-1-microbial-growth-13-728.jpg?cb=1343361108

Temperature Growth Classifications, Continued



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3. Mesophile:
   - » 25- 40°C
   - » Most common
   - » Human pathogens
4. Thermophile
5. Hyperthermophile/extreme thermophile:
   - » Archaea
   - » Producers using Sulfur, not light & CO_2

Fig 6.1Growth Rate of different groups in response to temp

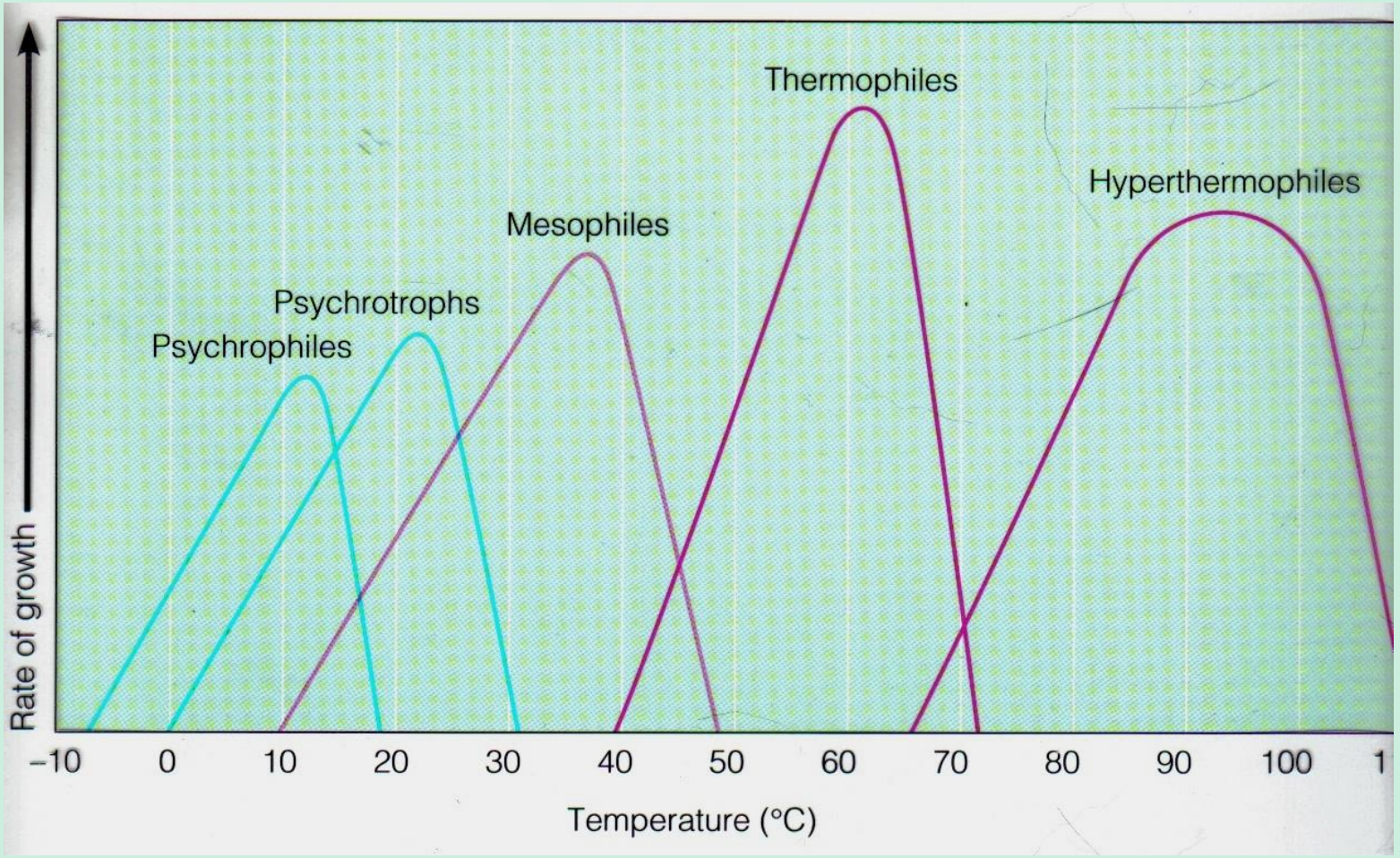


Fig 6.2 Food Spoilage Temperatures

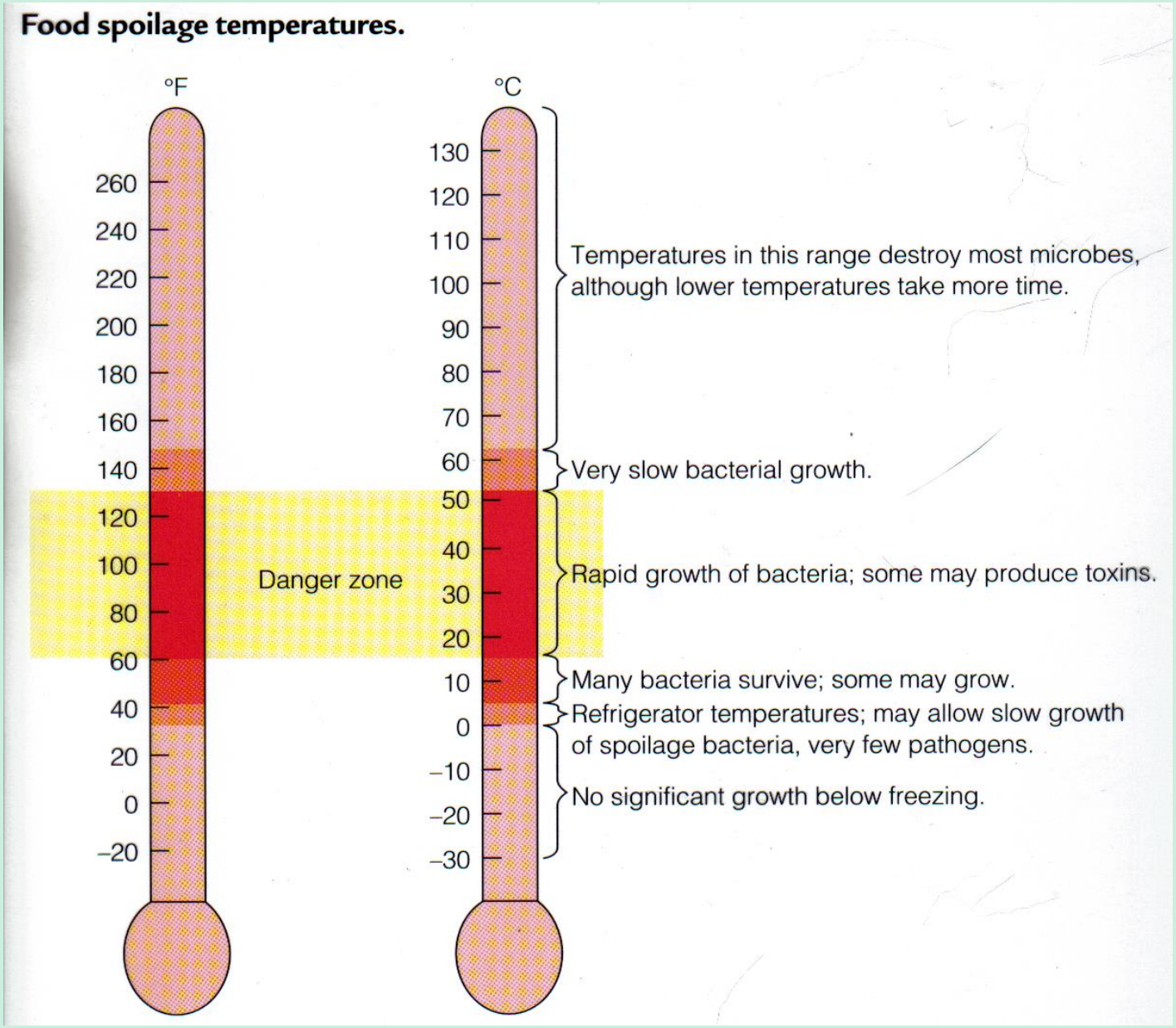
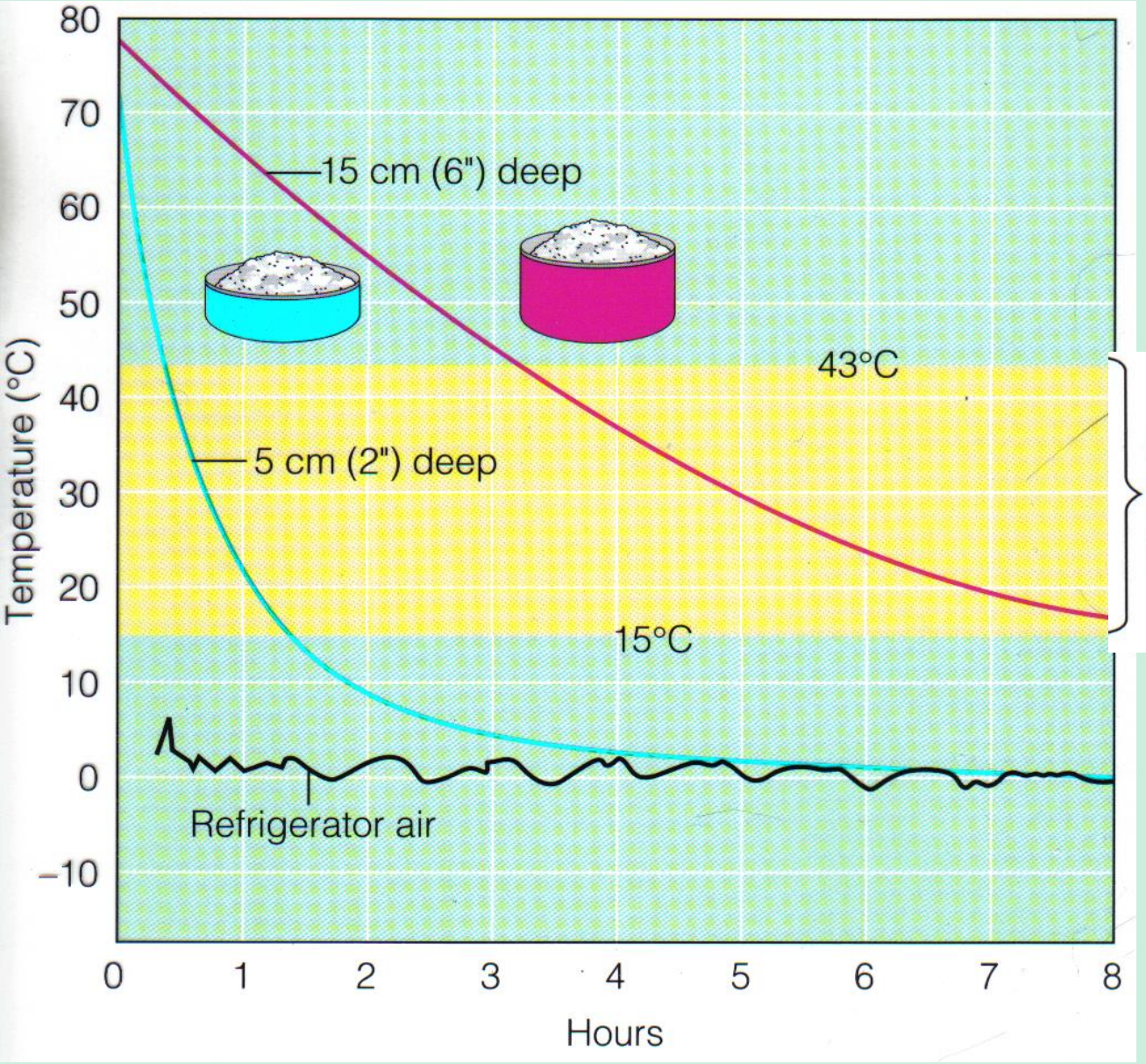


Fig 6.3 Effect of food amount on its cooling rate & spoilage chance



Approximate temperature range at which *Bacillus cereus* multiplies in rice

2. pH

pH

A. Most bacteria need a pH of 6.5- 7.5

   i. Foods preserved acidically avoid spoilage longer

B. Acidophiles

C. Buffer: Substance that resists changes in pH when acid or base added

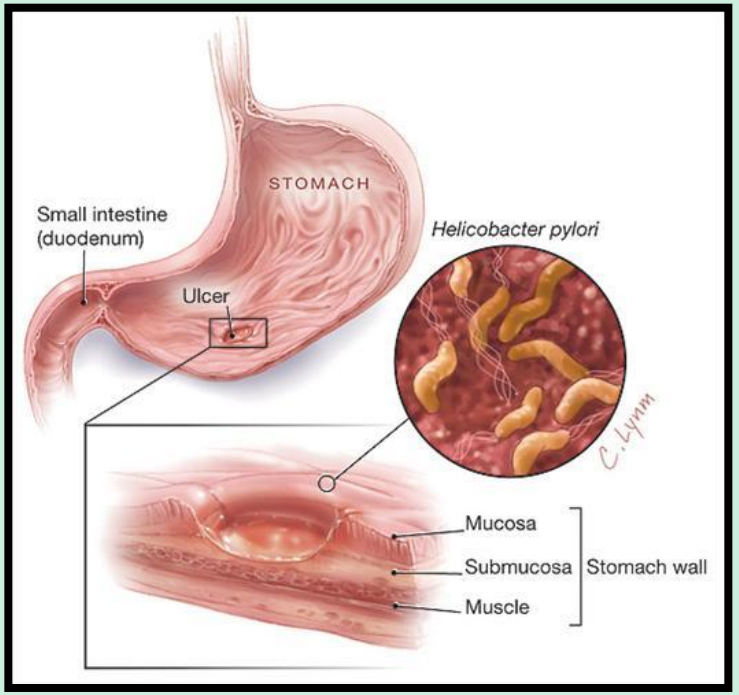
   i. neutralize growth by-products

   ii. Examples: peptones, phosphate salts, amino acids

Acidophiles (*Helicobacter pylori*)

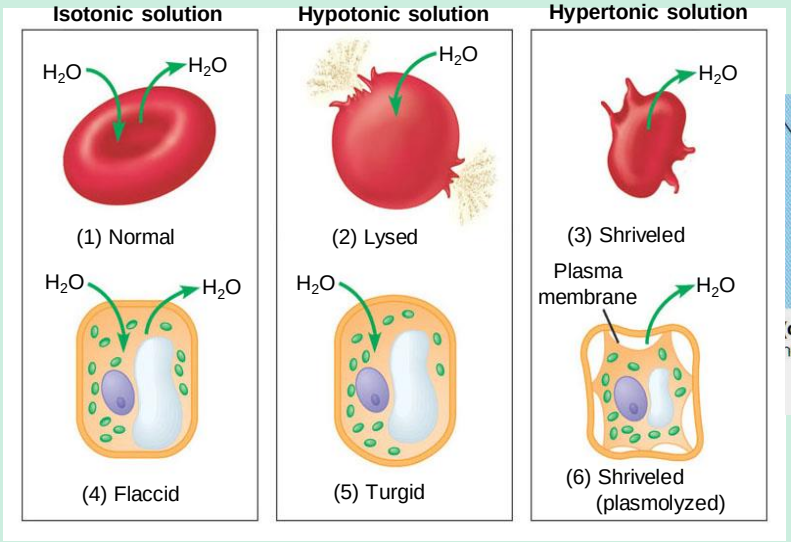
optimum in pH range 0-5.5

Acidophiles are found in sulfuric pools and geysers, areas polluted by acid mine drainage and even our own stomachs.



Osmotic Pressure

3. Osmotic Pressure
   - A. Hypertonic solution
     - i. Cell wall DOESN'T protect
     - ii. Plasmolysis occurs: dehydration, membrane pulls away & growth inhibited
     - iii. Food preservation with ⇑ sugar or salt: honey, jam, salted meat
     - iv. Making media- need proper ratio/enough water in agar or growth inhibited
   - B. Hypotonic Solution
     - i. Cell wall DOES protect
     - ii. Cell wall limits water intake & prevents osmotic lysis



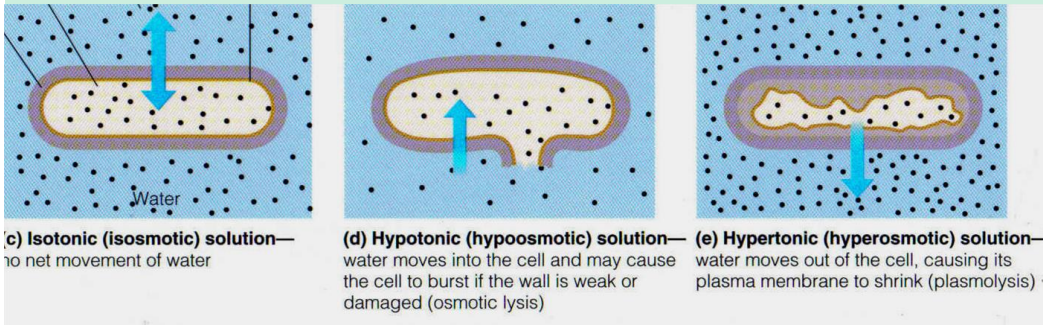
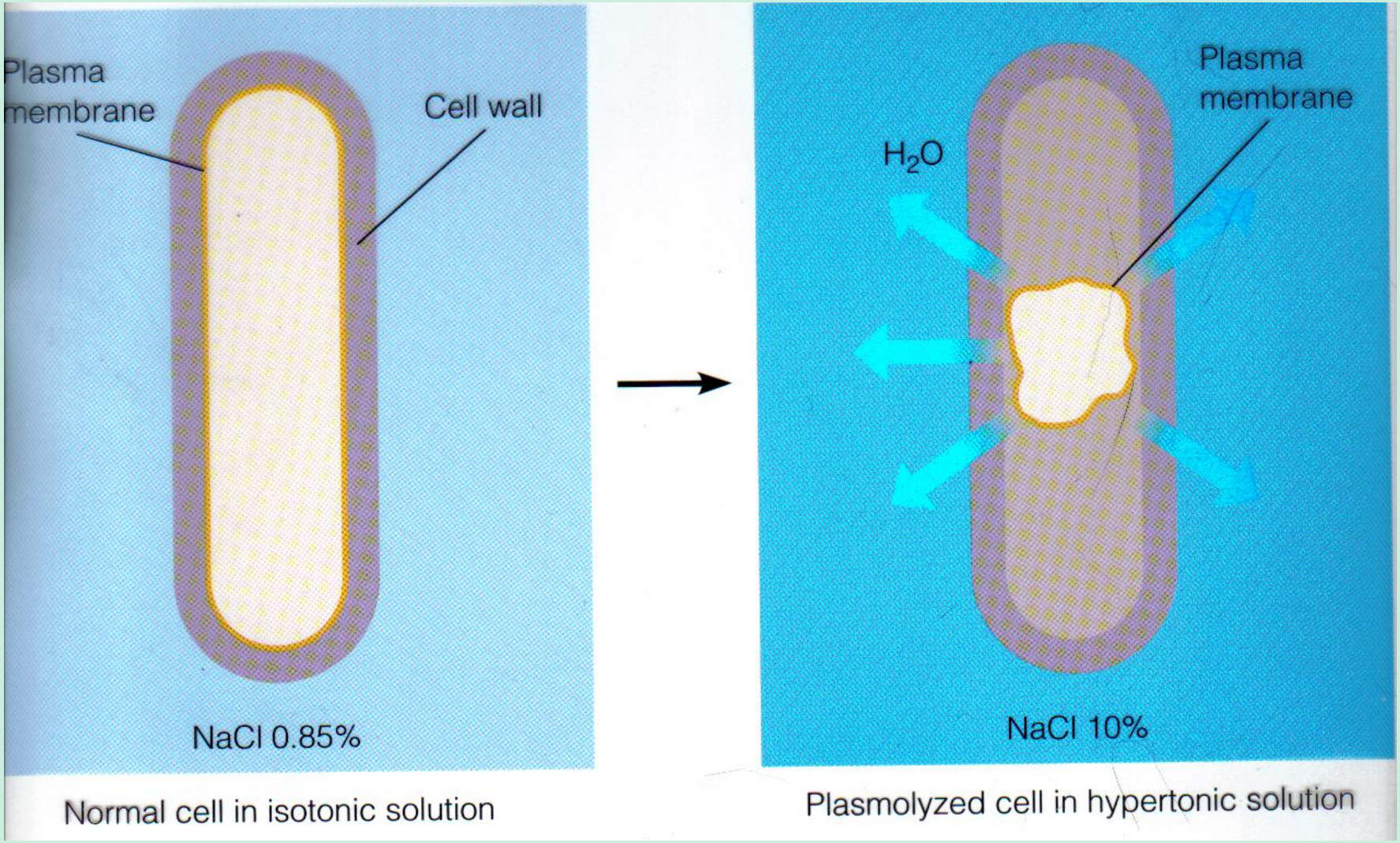
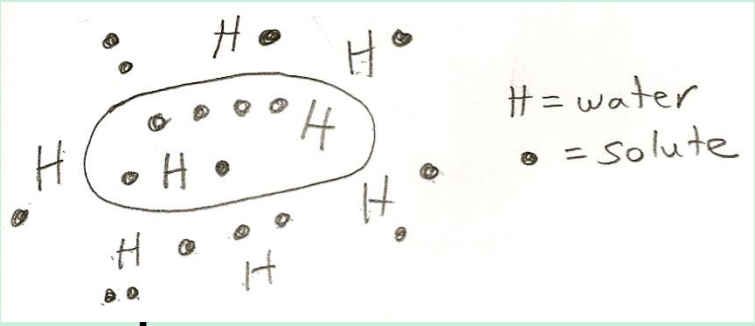


Fig 6.4 Plasmolysis



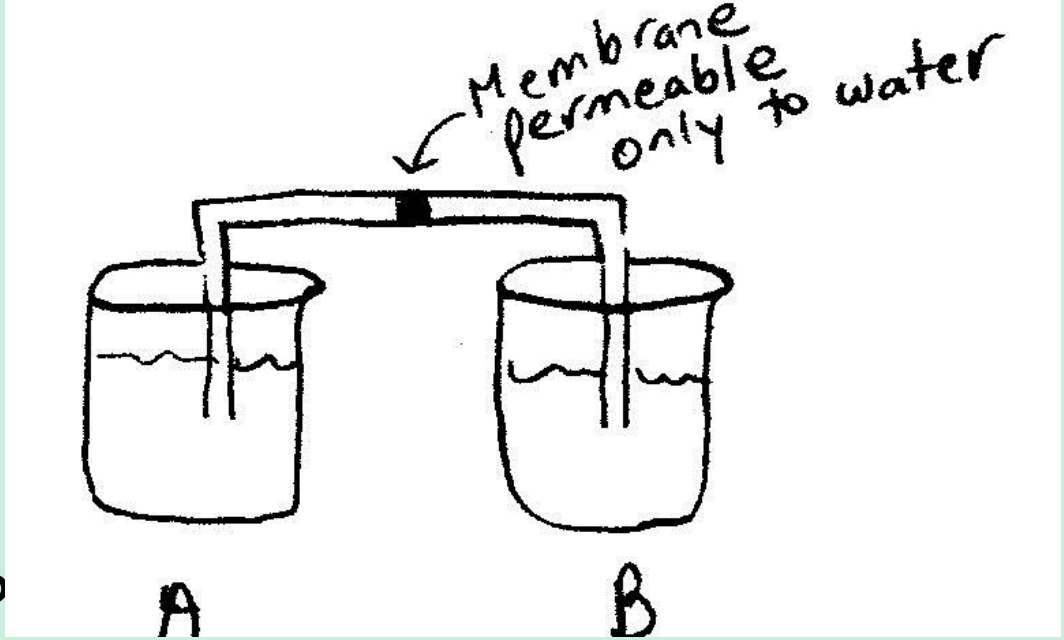
Miscellaneous Review

1. Purpose of buffer? Why needed?



2. Diagram above:
   A. Type of environment?
   B. Direction of osmosis?
   C. Result or effect on cell?
3. Ingredient that indicates complex media?
4. 2 reasons pickles resist spoilage?
5. 5 cells with a generation time of 20 minutes are grown for 2 hours. How many cells now?
6. Lab #10-12 Table 12.1: Complex vs. defined?

Osmosis Review



What if?

1. 1% NaCl vs. 2% NaCl
2. 2% NaCl vs. 2% NaCl
3. 2% NaCl vs. 2% Sucrose
4. 1% NaCl vs. 2% Sucrose
5. 2% Glucose vs. 2% Sucrose
6. 2% Lactose vs. 2% Sucrose

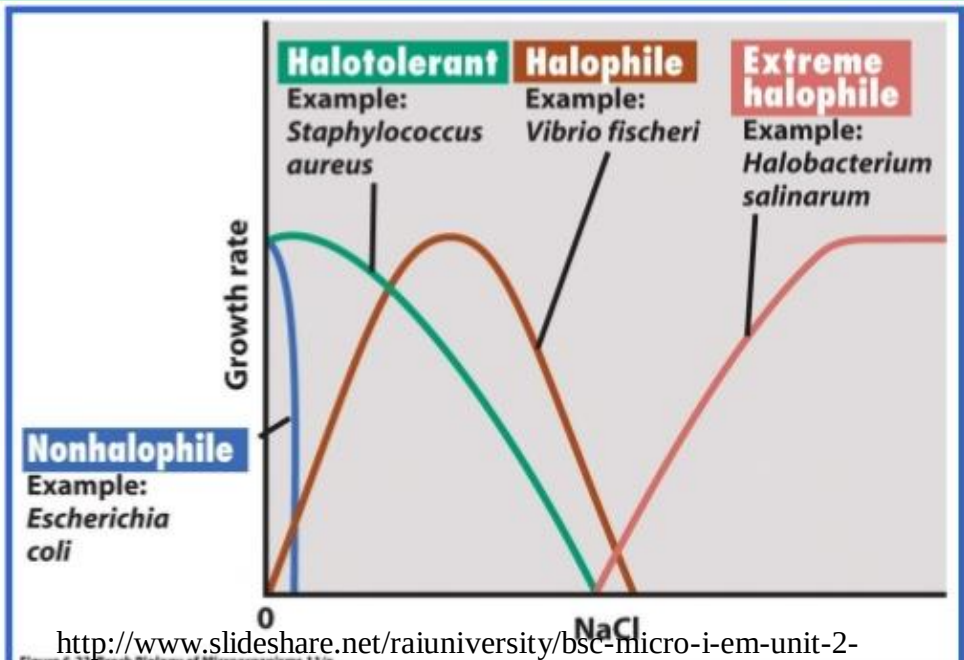
Halophiles

C. **Extreme/obligate** halophile
   i. Requires extreme salt, 20-30%
   ii. Dead Sea & Great Salt Lake
   iii. Archaea

D. **Facultative** halophile
   i. CAN grow w/ higher than normal salt, but prefers normal salt levels
   ii. Facultative always means: CAN grow, BUT prefers the opposite

Picture is Mannitol Salts Plate

- MRSA & other *Staph aureus* are facultative halophiles.
- Can be differentiated from other *Staph* species.



http://www.slideshare.net/raiuniversity/bsc-micro-i-em-unit-2-

Videos

https://www.youtube.com/watch?v=S3zOLwCsORw

https://www.youtube.com/watch?v=M83YOyfMyLg

https://www.youtube.com/watch?v=gWkcFU-hHUk

https://www.youtube.com/watch?v=VK-_YHakvho

Review Questions:

1. What are the two requirement categories for microbial growth?

2. What three factors influence physical growth?

3. What is a cold loving organism called?

4. What temperature category do most human pathogens occupy?

Review Questions:

5. To what temperature category do Archaea belong?

6. What is the pH range most bacteria require for growth?

7.What type of solution causes plasmolysis?

8). What is plasmolysis?

Chemical Requirements

Chemical Requirements (CHONPS)

1. Carbon – ALL types of organic compounds in the microbe
2. Nitrogen:
   A. Amino acids (proteins)
   B. Nitrogen bases (DNA, RNA)
3. Phosphorus
   A. ATP, membrane phospholipids, DNA
4. Sulfur
   A. Amino acids (proteins)
5. Oxygen
   A. Cellular respiration (energy production)
   B. BUT the by-products of respiration can be toxic

C. Superoxide free radical: $O_2^{-}\bullet$ Toxic oxygen forms

i. Toxic by-product of aerobic respiration

ii. ALL organisms & cells must get rid of

iii. Eukaryotic lysosomes in phagocytes intentionally contain $O_2^{-}\bullet$ & the high concentrations are used to kill engulfed bacteria

D. Steps to break down $O_2^{-}\bullet$ & survive:

i. Superoxide dismutase (SOD), an enzyme

$$O_2^{-}\bullet + O_2^{-}\bullet + 2H+ \rightarrow H_2O_2 + O_2$$

ii. Peroxide ion (O_2^{2-}) of H_2O_2 is toxic

iii. Break down H_2O_2 w/1 of 2 enzymes:

» Catalase: $2H_2O_2 \rightarrow 2H_2O + O_2$

» Peroxidase: $H_2O_2 + 2H+ \rightarrow 2\,H_2O$

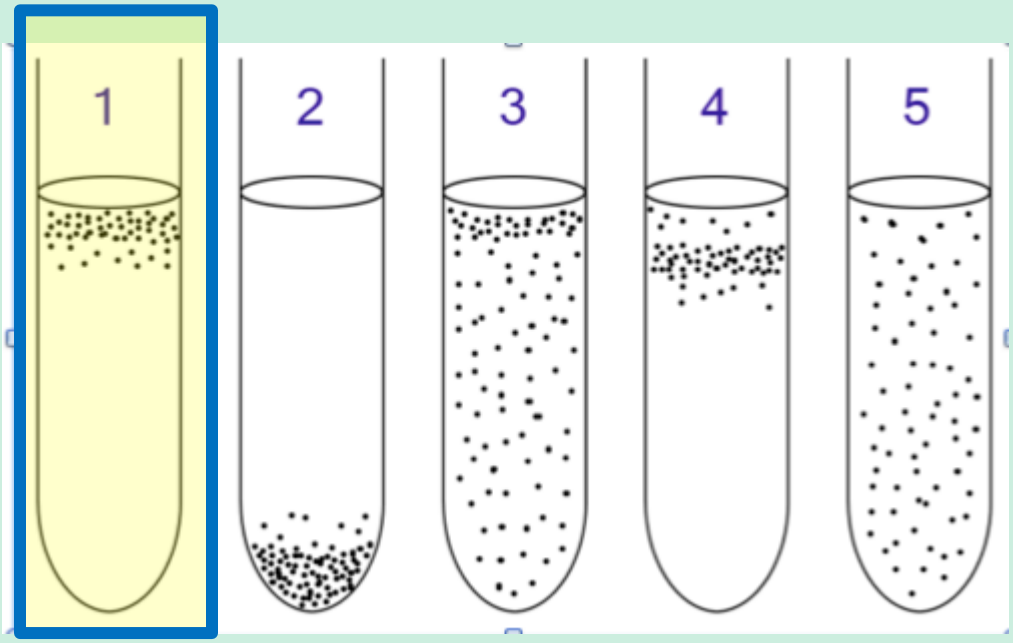
Videos

https://www.youtube.com/watch?v=dlZ5ROca0KI

https://www.youtube.com/watch?v=ozJeehsCG7Q

Oxygen Classifications

1. **Obligate aerobes**: REQUIRE O2
   A. Have SOD & catalase, which neutralizes toxic forms of oxygen.
   B. Oxygen can be used.



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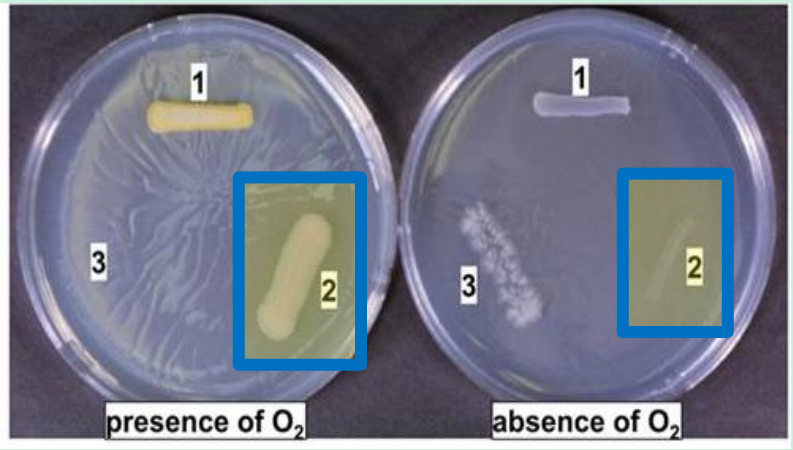

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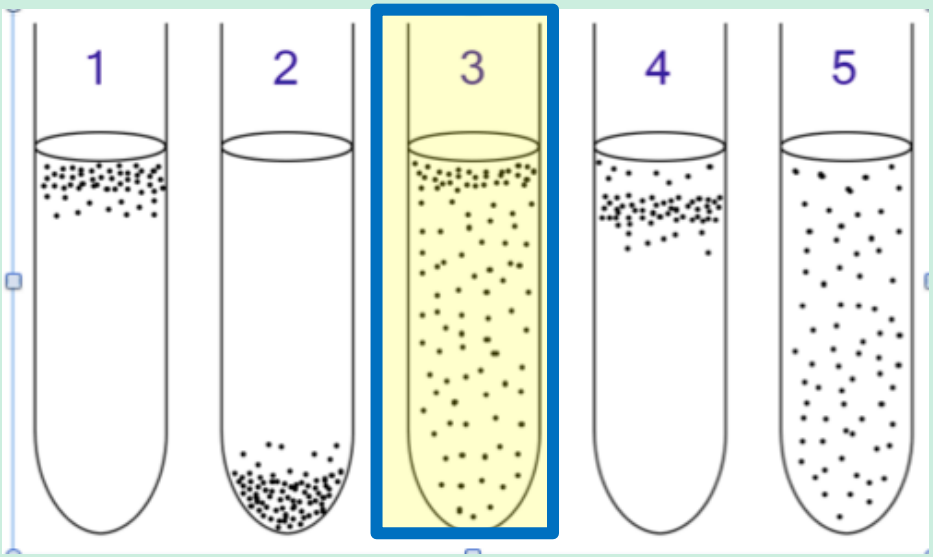
Oxygen Classifications

2. **Facultative anaerobes**: CAN grow w/o O2, but grows best (⇑ energy production) w/O2
   A. WithOUT O2; fermentation, anaerobic respiration, end products are alcohols or acids that still have bonds with energy. (NOT broken all the way to CO2)
   B. Have BOTH SOD & catalase to neutralize toxic by-products of cellular respiration.
   C. More energy production (ATP) and faster growth in presence of oxygen.



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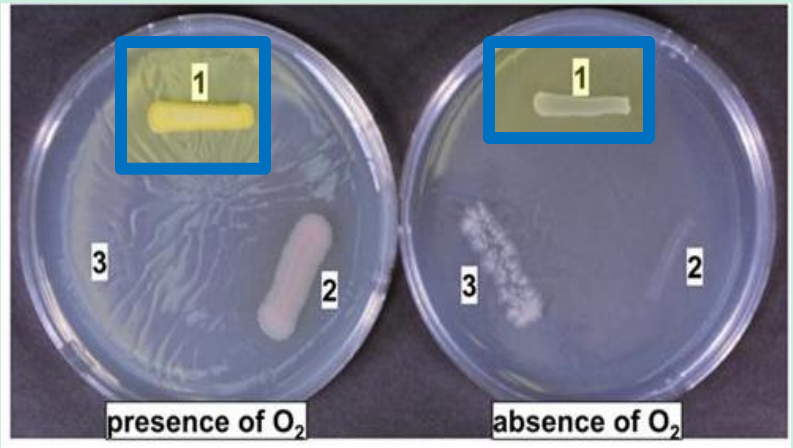

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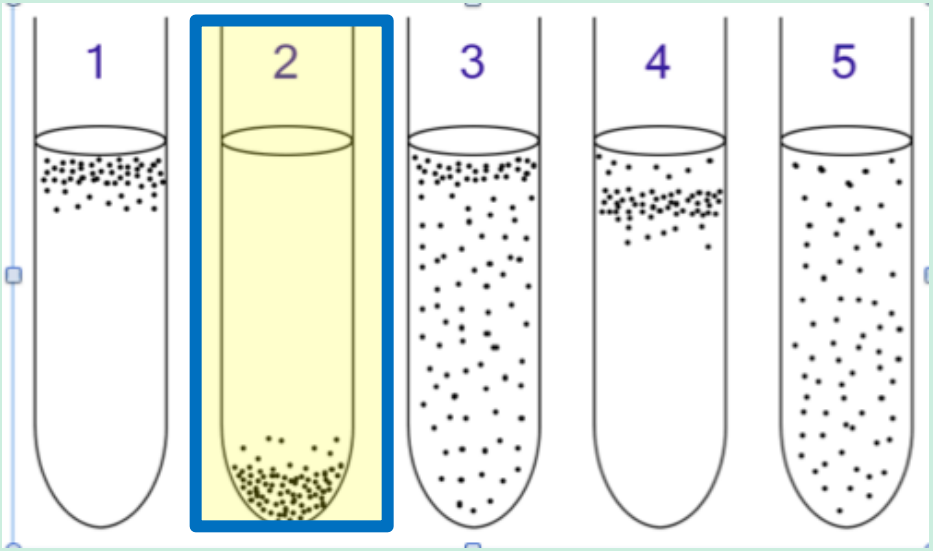
Oxygen Classifications

3. **Obligate anaerobes**: Molecular/atmospheric O2 is toxic
   A. Must have oxygen, but DOESN'T use atmospheric O2.
   B. INSTEAD, gets oxygen from compounds.
   C. Lack both SOD & catalase.
   D. Oxygen is toxic.
   E. Exposed too long to oxygen and death occurs due to build up of super-oxide free radical.



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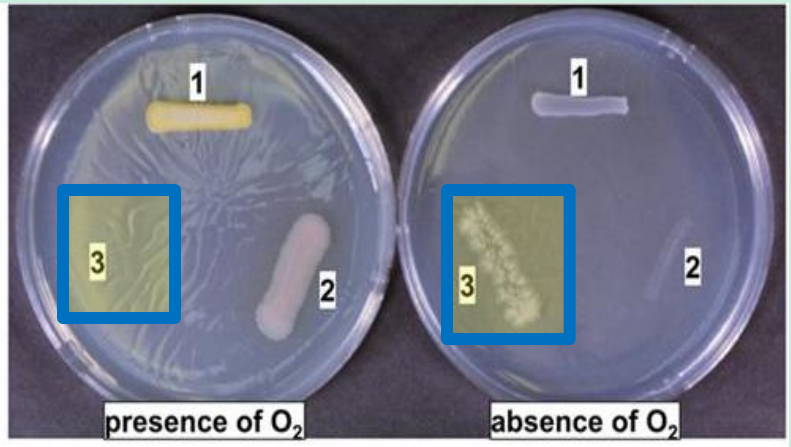

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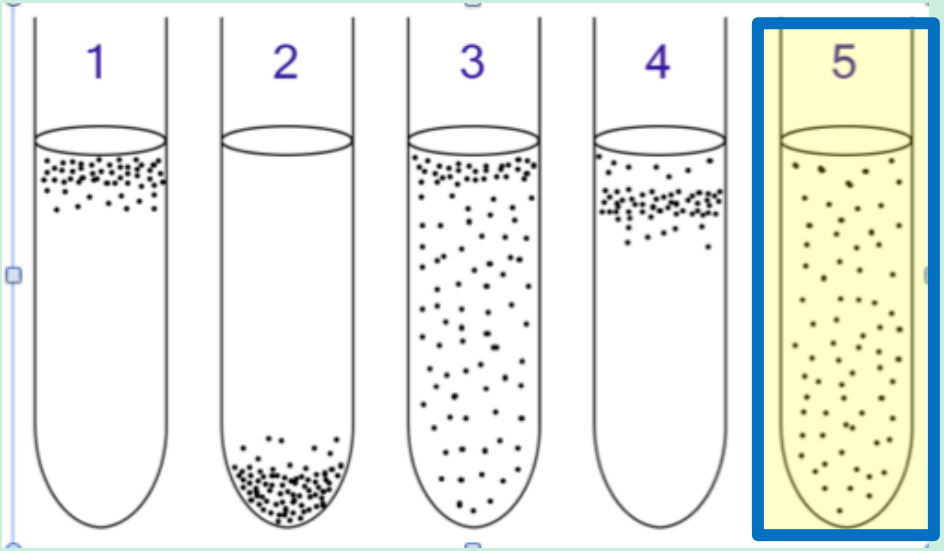
Oxygen Classifications

4. **Aerotolerant anaerobes:** CAN grow w/O2, but prefer no O2
   A. Contain SOD, but no catalase
   B. Partially neutralize toxic forms of oxygen. Can TOLERATE oxygen.
   C. More energy, ATP, produced in anaerobic conditions.



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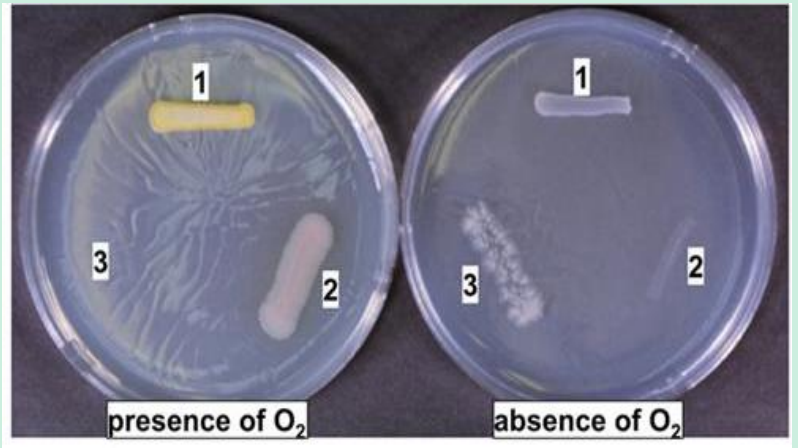

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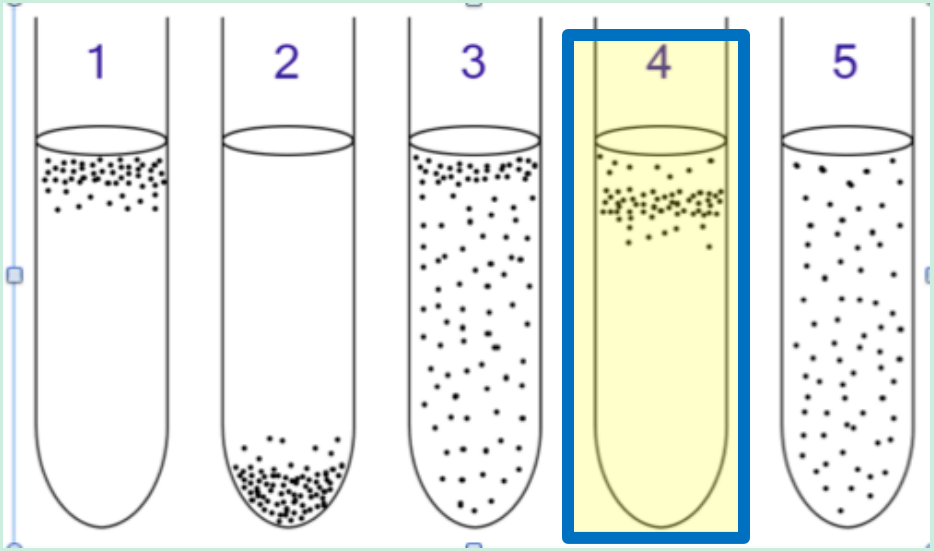
Oxygen Classifications -Microaerophile

5. **Microaerophiles:** Require O2, but in lower [ ] than air
   A. Have both SOD & catalase, but at lower levels
   B. If exposed to normal levels of oxygen, doesn't have enough SOD & catalase to neutralize the amount of toxic forms of oxygen.



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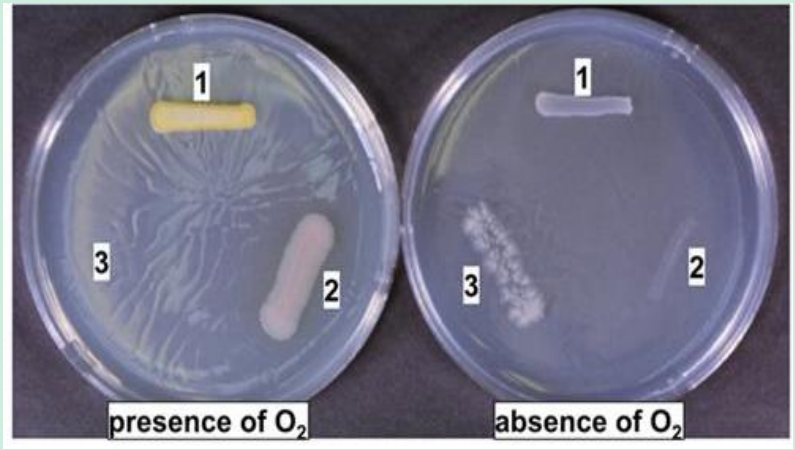

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Table 6.1 Effect of Oxygen on Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaero-philes
Effect of oxygen on growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial growth in tube of solid growth medium					
Explanation of growth patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of oxygen's effects	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Actual Tubes – Interpret Oxygen Requirements

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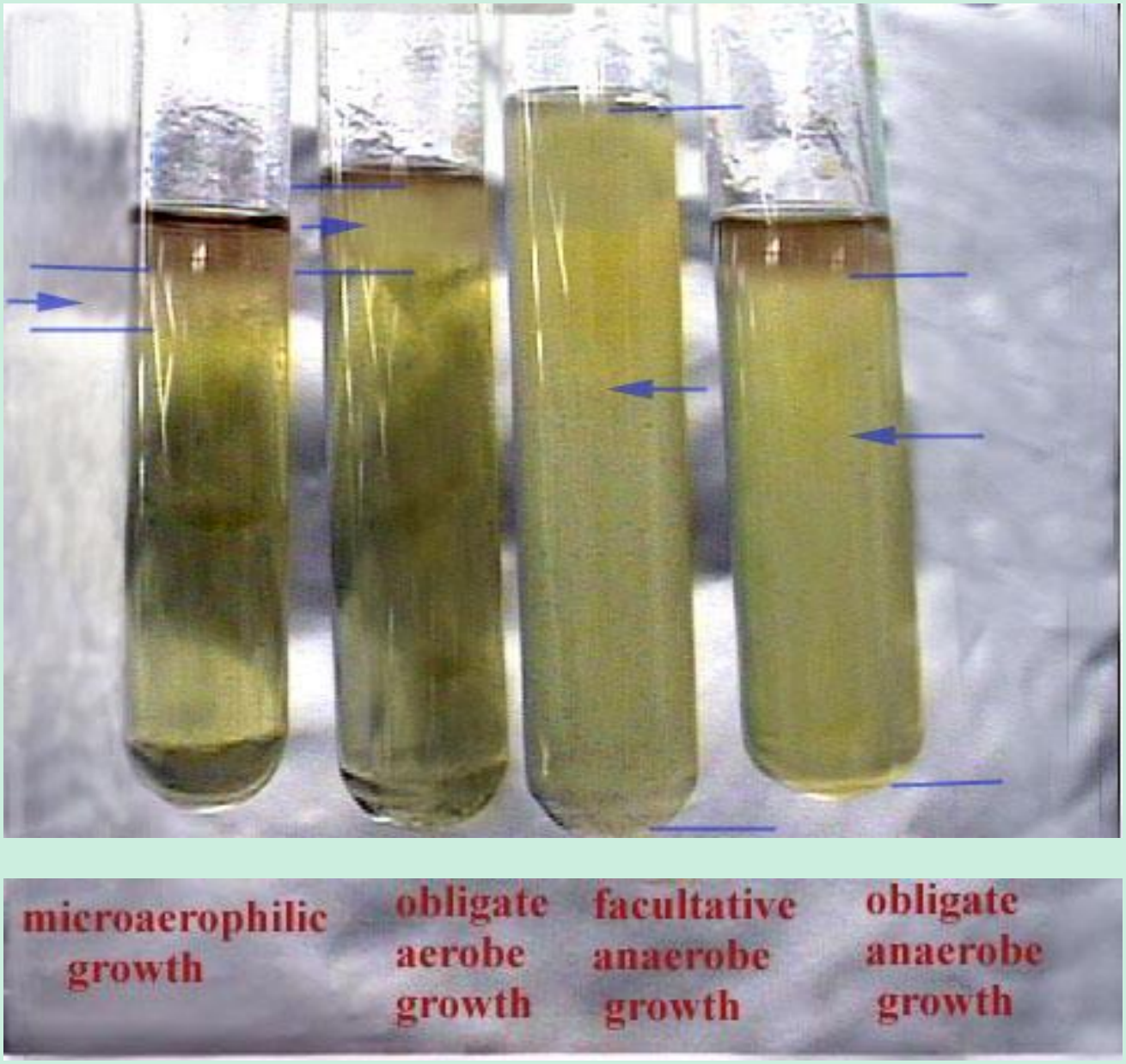


Review: Chemical Requirements for Growth

1. What are the chemical requirements for microbial growth?

2. What two molecules does nitrogen compose?

3. What chemical requirement(s) is(are) found notably in membrane phospholipids?

4. What biological molecules contain sulfur?

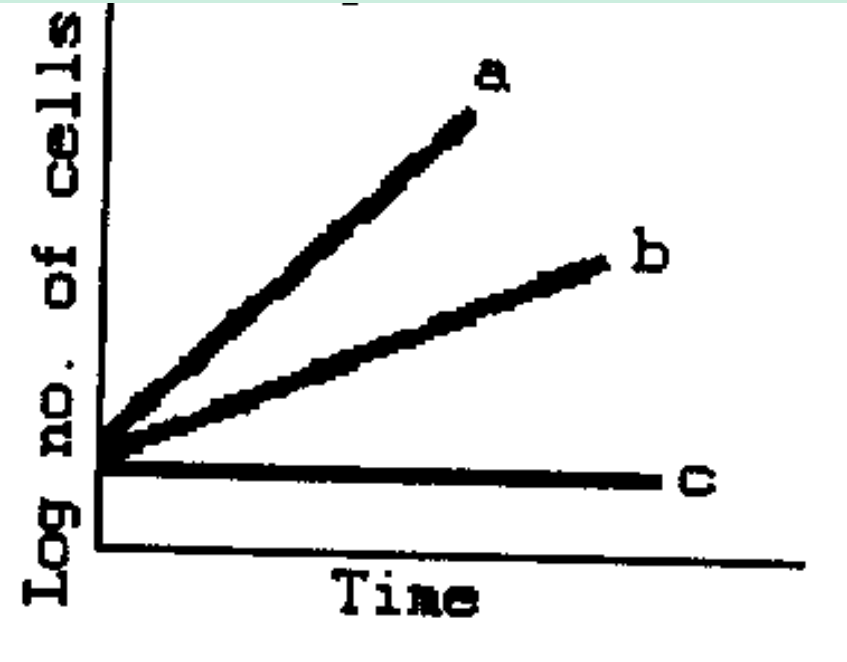
Review: Chemical Requirements for Growth

5. What process requires oxygen in living organisms?

6. What enzyme turns superoxide free radicals into hydrogen peroxide?

7. What are the two enzymes that can break down hydrogen peroxide into non-harmful substances?

Graph Example Problems



- Which line is a facultative anaerobe grown anaerobically?
- Facultative anaerobe in presence of oxygen?
- Psychrotroph at room temp?
- Psychrotroph in the refrigerator?

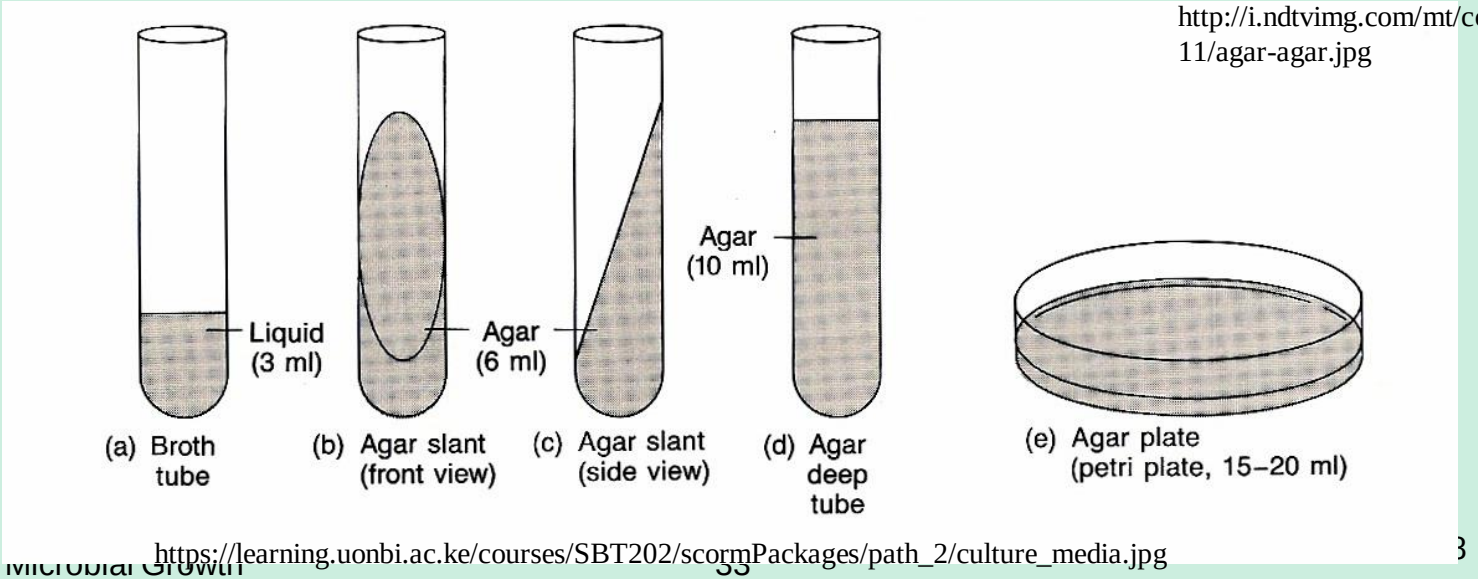
Terms

Culture Medium

1. Culture medium
2. Agar:
   A. Solidifying agent only
   B. Seaweed polysaccharide
   C. Melts at 100C, solidifies 40C
3. Plates
4. Slants
5. Deeps: Tube w/flat surface
   A. Motility
   B. Decreased O2 at bottom (Anaerobes won't grow, but facultative anaerobes will – TSI)

http://www.infosamak.org/english/admin_images/algue-palmaria-palmata.jpg

http://i.ndtvimg.com/mt/cooks/2014-11/agar-agar.jpg



https://learning.uonbi.ac.ke/courses/SBT202/scormPackages/path_2/culture_media.jpg

Terms Continued

6. Sterile: Contains no living organisms
   A. Autoclave: Steam under pressure
      i. 121C at 15psi for 20 minutes
7. Inoculum
8. Colony
9. Culture
10. Pure Culture
11. Streak Plate
    A. To obtain isolated colonies

http://blogs.fit.edu/wp-content/uploads/2014/05/Florida-Tech-Streaking-1.jpg

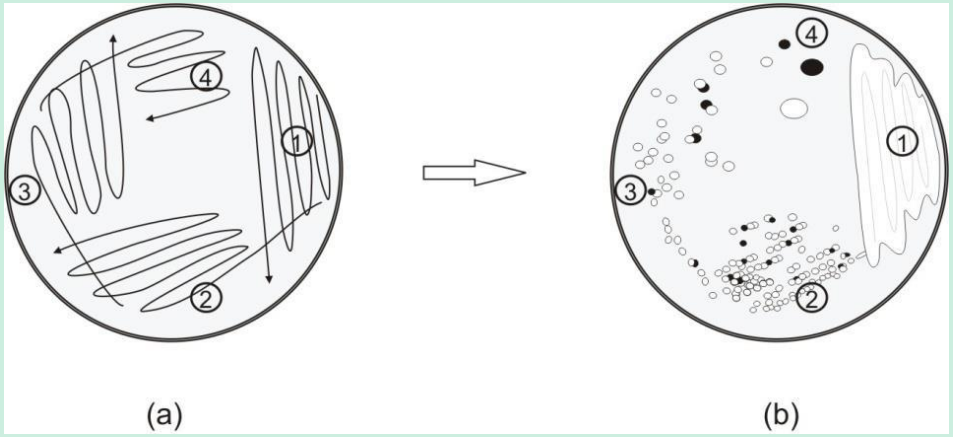
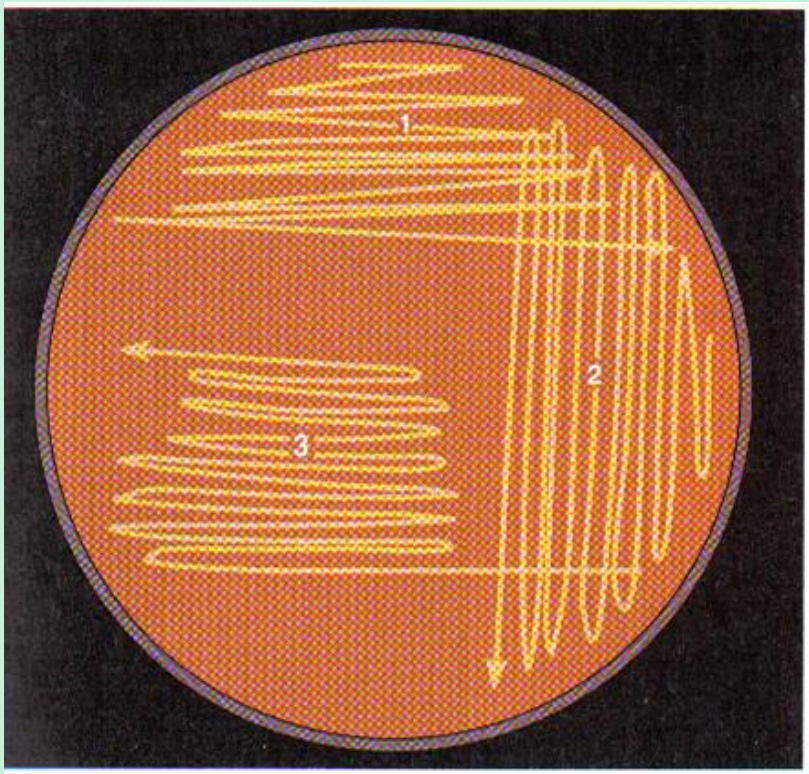
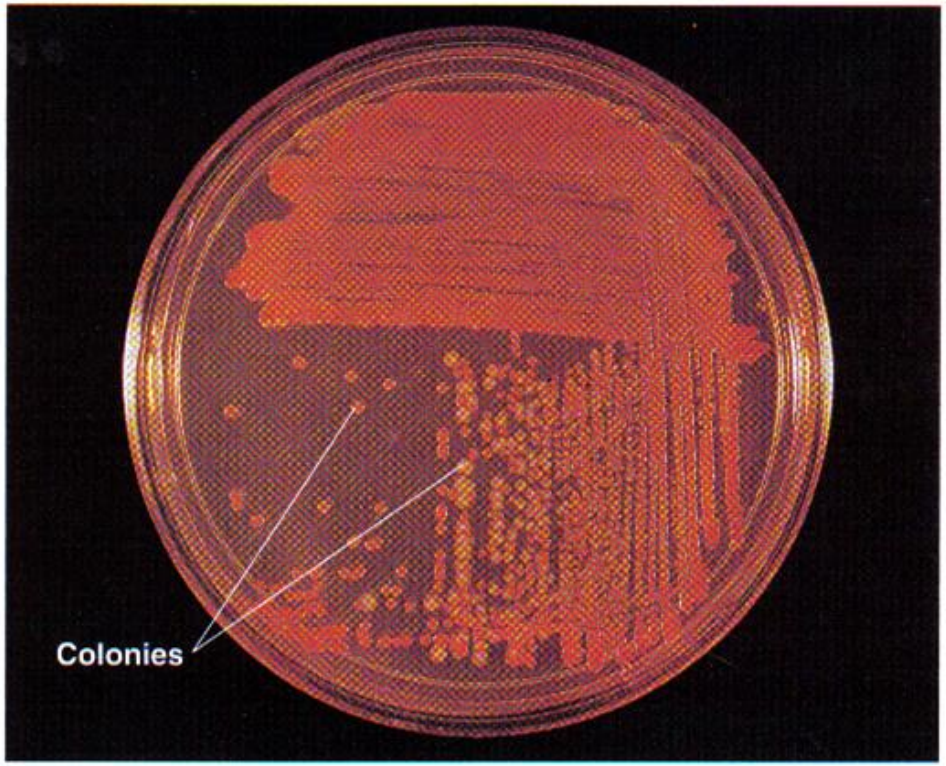


Fig 6.10 Streak Plate



(b)



Chemically Defined vs. Complex Media

12. Chemically defined medium
    A. Exact composition (formulas & amounts) known
13. Complex Media
    A. Exact composition unknown
    B. Extracts of yeast, meat, peptones (protein digests), blood….
    C. AKA "Nutrient" media

table 6.2 *A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as* E. coli

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

table 6.4 *Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria*

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Anaerobic Media/Methods – Reducing Media, Thio

1. **Reducing media**

   A. Ingredients combine with & reduce O2

Example of a Reducing Media:

Thioglycollate Broth

- Sodium thioglycollate combines with the oxygen producing water
- Agar: A small amount of agar thickens the broth to slow diffusion of oxygen
- Oxygen Indicator: Dye is pink in presence of excess O2, indicating how far oxygen has diffused into the tube
- Heat tubes prior to use to drive off absorbed O2 (Warm solutions cannot hold as much oxygen.)

http://www.tgw1916.net/images/CIMG6712_thioglycolate.jpg

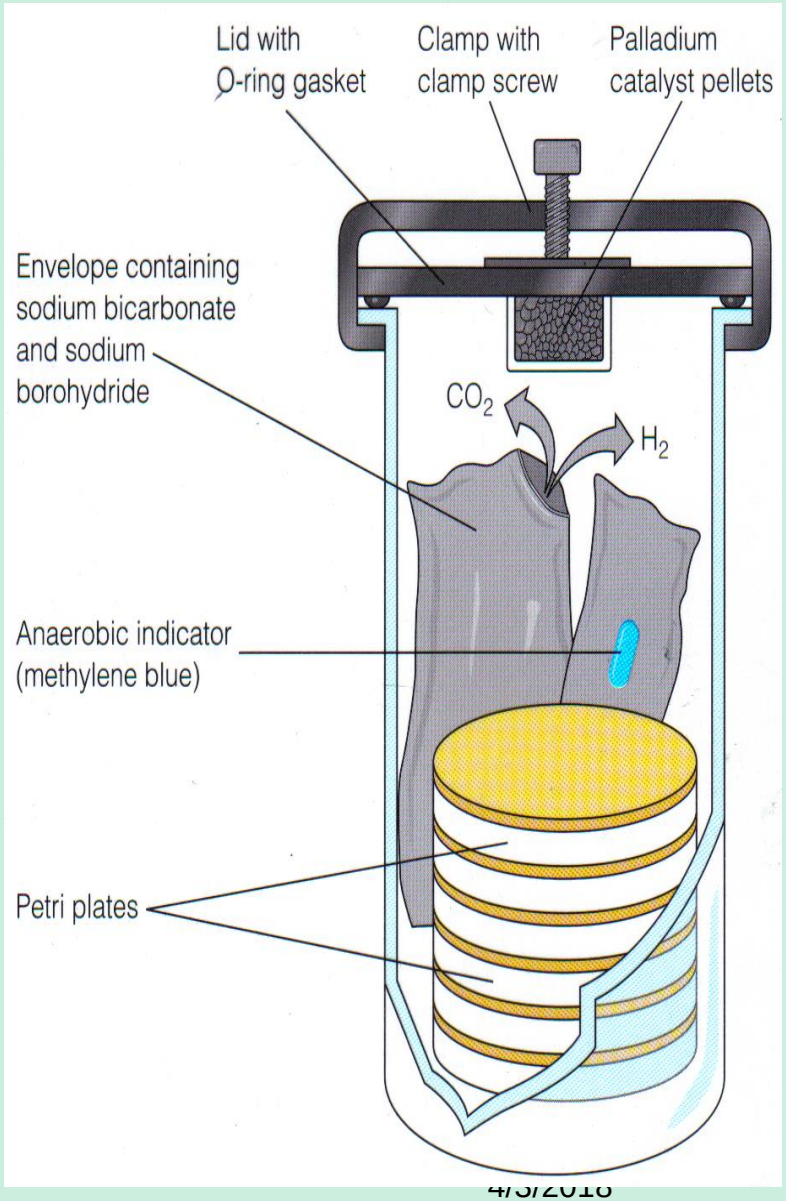
Anaerobic Media/Methods - Jars

2. **Anaerobic Jar** – 2 main types

A. Traditional: H2 generator & Palladium catalyst (like Organic) H2 + O2 → H2O. Jar becomes moist inside as reaction occurs.

B. AnaeroPack: Packet does two things – rapidly absorbs O2 & generates CO2

Fig 6.5 Anaerobic Jar



Anaerobic Media/Methods – Chamber/Hood

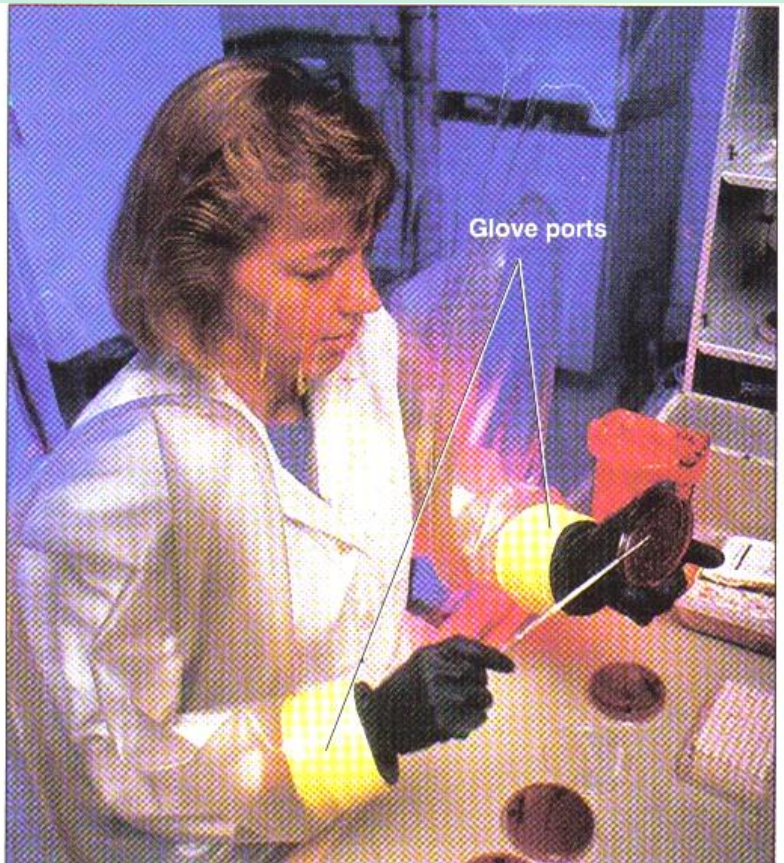
3. Anaerobic Chamber/Hood

A. O2 removed

B. Chamber filled with an inert gas like N2

C. Glove ports used to manipulate plates

Fig 6.6 Anaerobic Chamber/Hood



Special Techniques – CO2 & Living Host Cells

1. CO2 Incubators, candle jars, packets for microbes requiring ⇑ CO2
   A. Do candle jars create an anaerobic environment?

2. Living host cells: Obligate intracellular bacteria. Example: leprosy

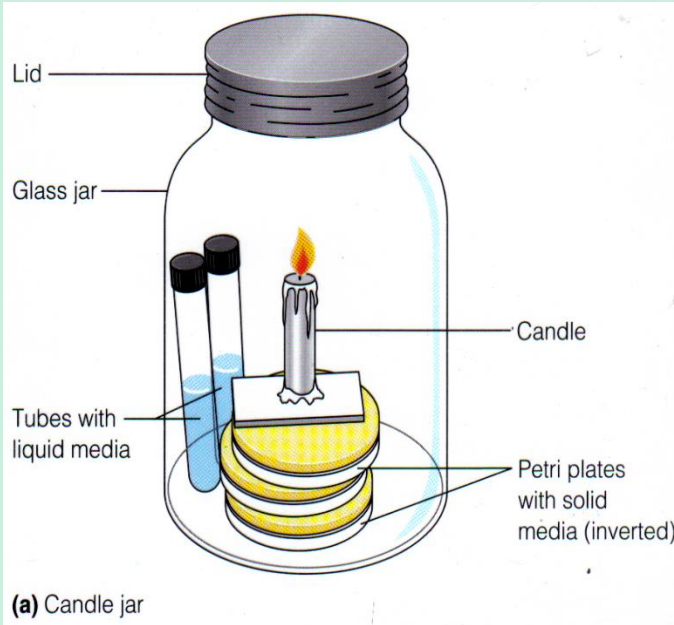
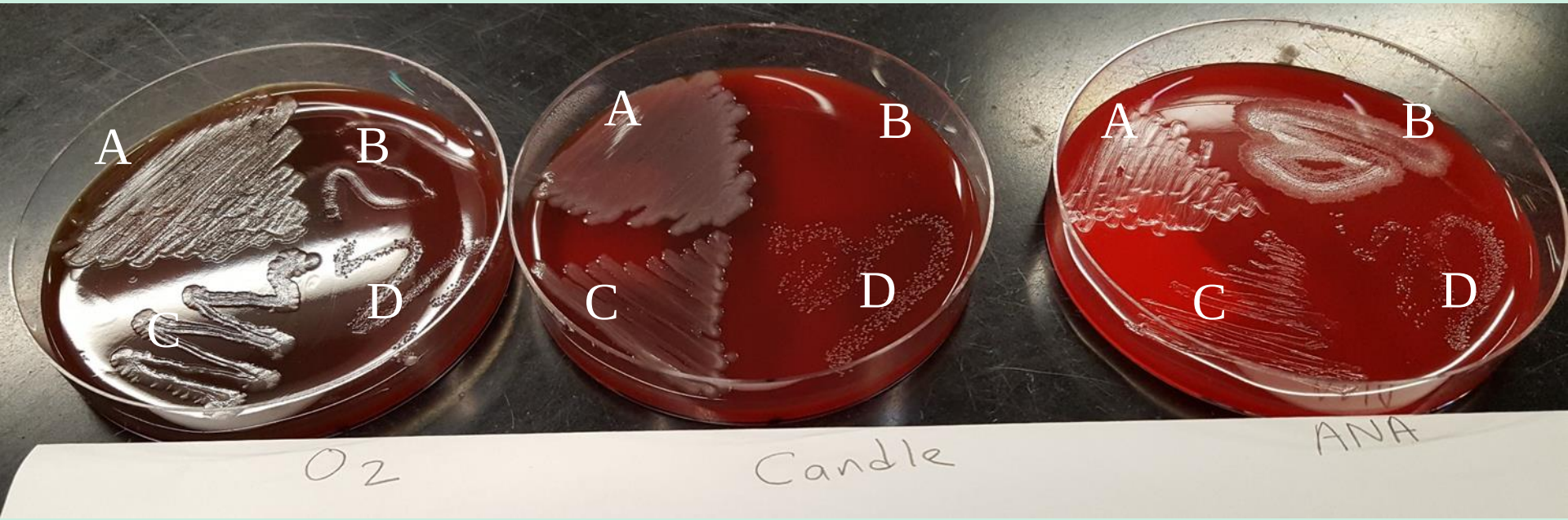


Fig 6.7 CO2 Rich Environments

http://www.dailymail.co.uk/health/articl

http://epi.ufl.edu/wp-content/uploads/2015/12/Armadillo-Close-Up.jpg

Growth Analysis



Using the labeled blood agar above, classify each organism's ability to use/not use oxygen and explain why you made that choice.

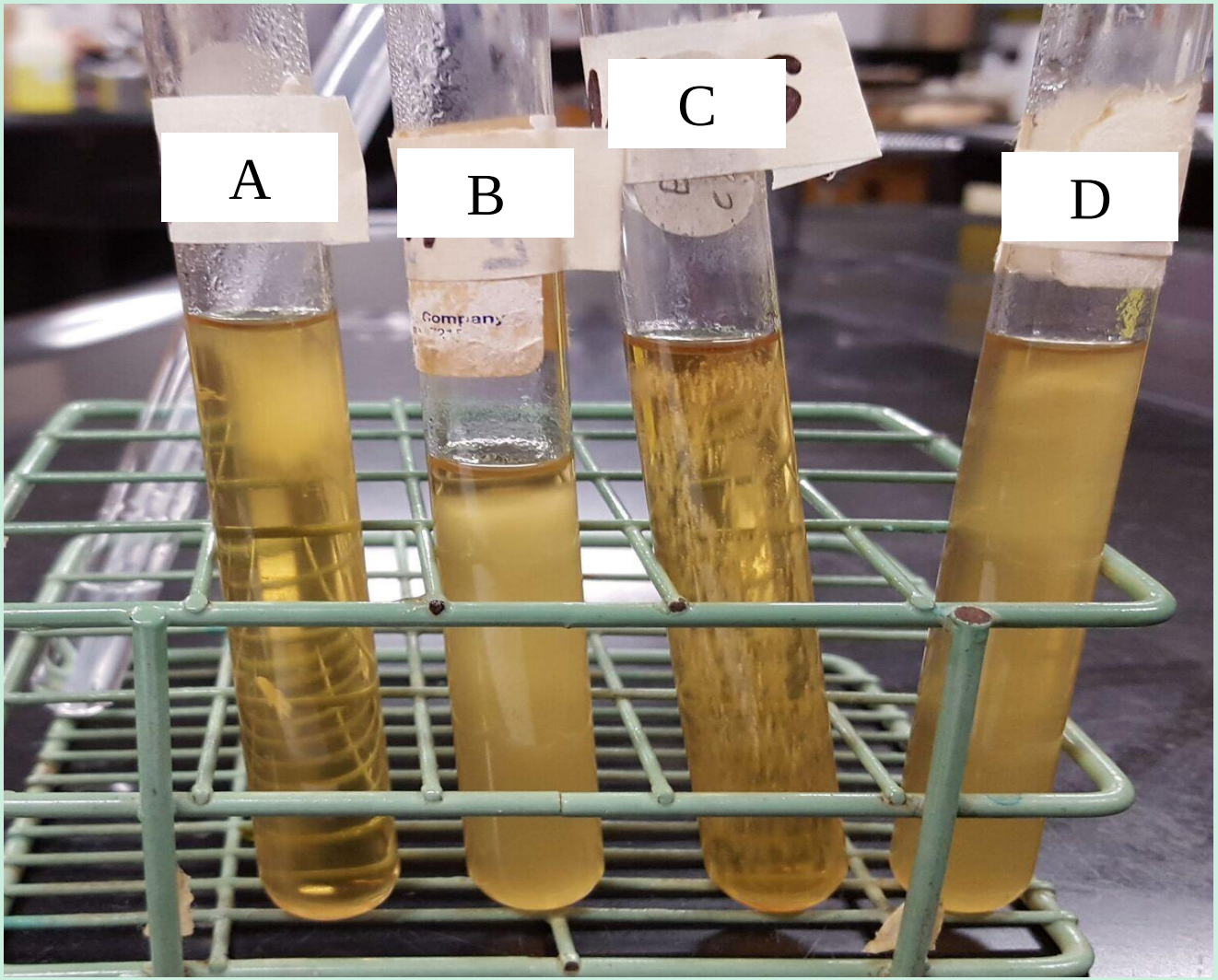
A =

B =

C =

D =

Growth Analysis



Using the Thio broths pictured to the left, classify each organism's ability to use/not use oxygen and explain why you made that choice.

PEA - Selective Media

3. Selective Media

A. Only certain groups grow

B. Due to antibiotics, salt, acidity, chemicals, etc

C. PEA (Phenylethyl alcohol)

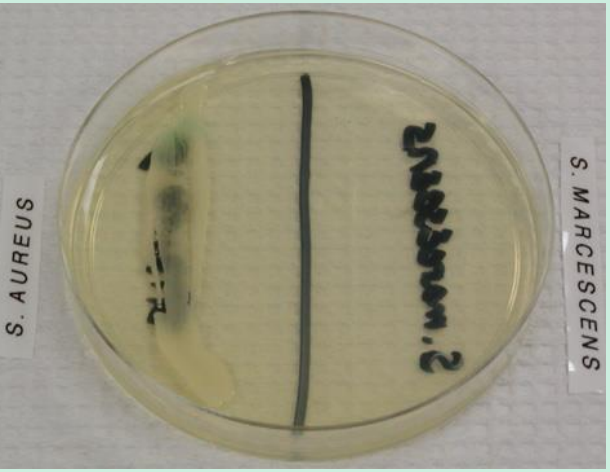
i. Selective for (allows growth) of GP

ii. Dissolves GN outer membrane (supposed to kill but may simply inhibit & have "breakthrough growth")

iii. If alcohol evaporates & at a lower concentration, what will happen?

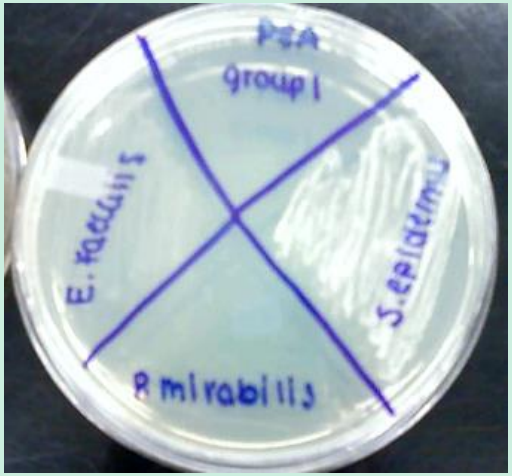
» "Breakthrough" GN growth (Compare growth to other plates)

iv. Like gram stain, if alcohol was higher content the alcohol would start to affect & inhibit GP also



http://www.austincc.edu/microbugz/images/PEA.jpg

https://c1.staticflickr.com/1/97/268073707_1ae79f934e.jpg



https://classconnection.s3.amazonaws.com/733/flashcards/695733/png/screen_shot_2011-10-22_at_2.28.35_pm1319308168786.png

BAP - Differential

4. Differential Media

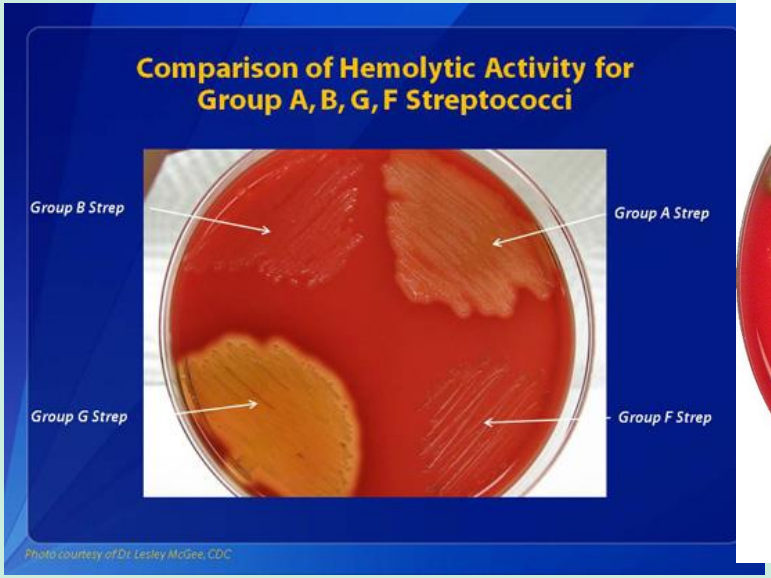
A. Ingredient used to intentionally cause appearance differences to distinguish 1 group/type from another

B. Blood agar

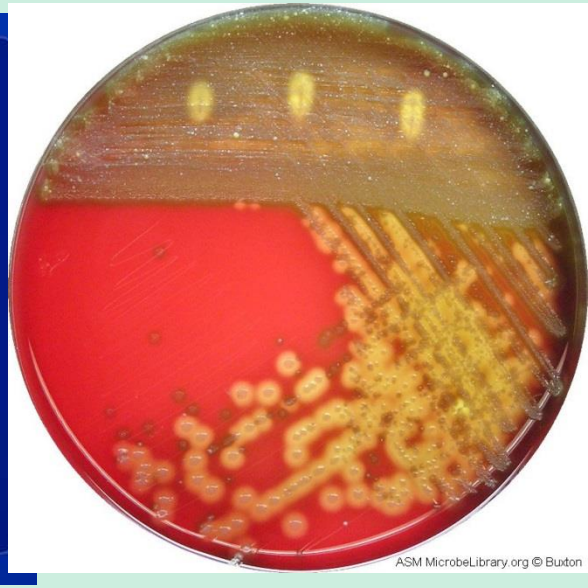
i. NOT selective – most bacteria will grow

ii. It IS differential based on hemolysis which indicates the organism has enzymes known as hemolysins

http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab14/images/asm_abg



http://www.cdc.gov/groupbstrep/images/lab-hemolytic-lg.jpg



http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab14/images/asm_mix

EMB - Differential & Selective Media

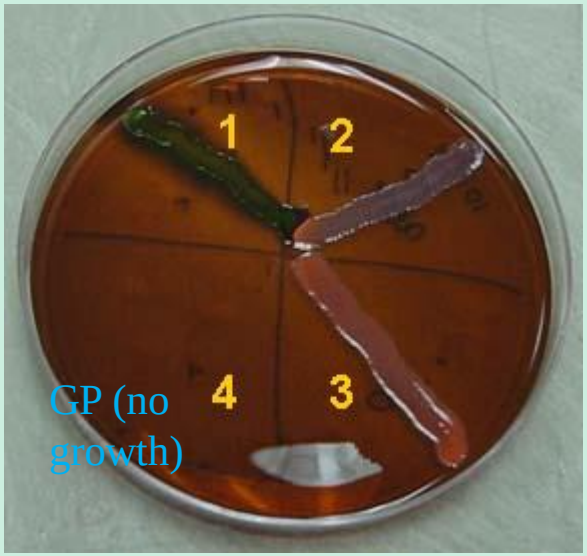
C. EMB (Eosin Methylene Blue)

i. Selective: The dyes methylene blue & eosin inhibit GP

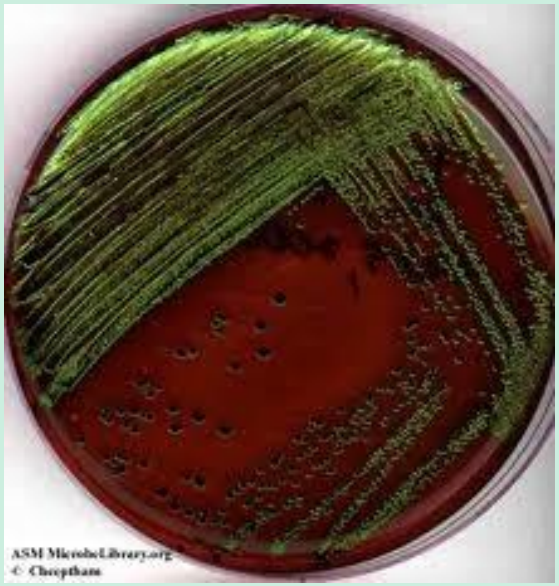
ii. Differential – contains lactose & a pH indicator that changes color due to acid production if lactose is used

» DARK Purple and/or metallic sheen: lactose fermenter

» No color change/is color of agar: Non-lactose fermenters (light color)



http://iws2.collin.edu/dcain/CCCCD%20Micro/EMBplate.jpg



http://o.quizlet.com/i/RKh6SRzEbi-JbGH_rXU0iA_m.jpg

"Treated" sewage

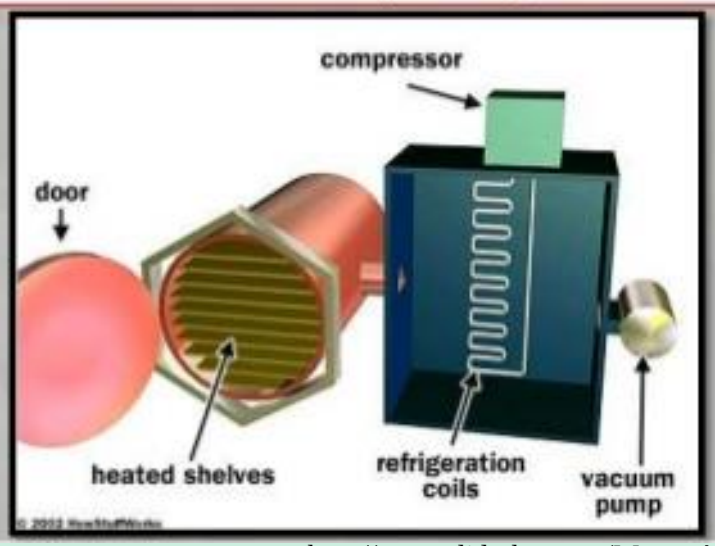
http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Coliform_assays/Plates_with_Colonies/Nine_Mile_STP_0.01mL_EMB_P8011340md.jpg

Preserving Bacteria

Preserving Bacteria

1. Refrigeration (short-term)
2. Deep-freezing
3. Lyophilization (freeze-drying)- long term

- In deep freezing a pure culture of microbes is placed in a suspending liquid and quick-frozen at temperatures ranging from -50°C to -95°C. (several years later)
- During lyophilization (freeze-drying), a suspension of microbes is quickly frozen at temperatures ranging from - 54°C to -72°C, and the water is removed by a high vacuum (sublimation).

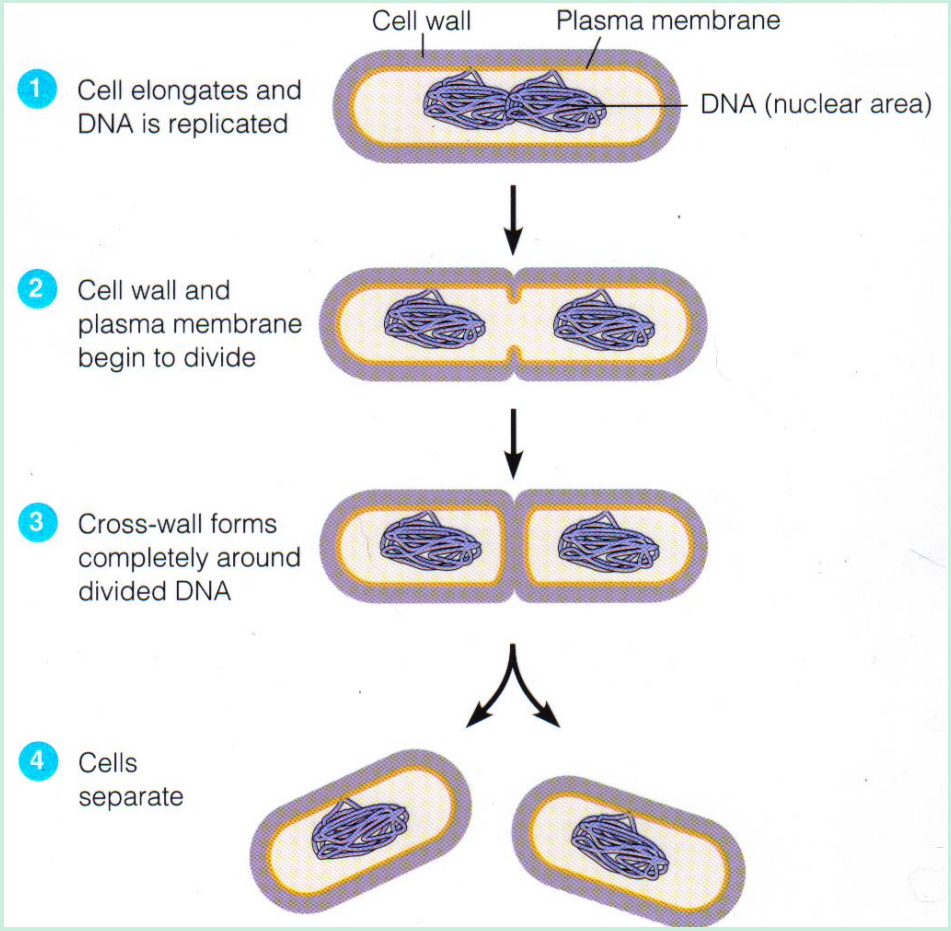


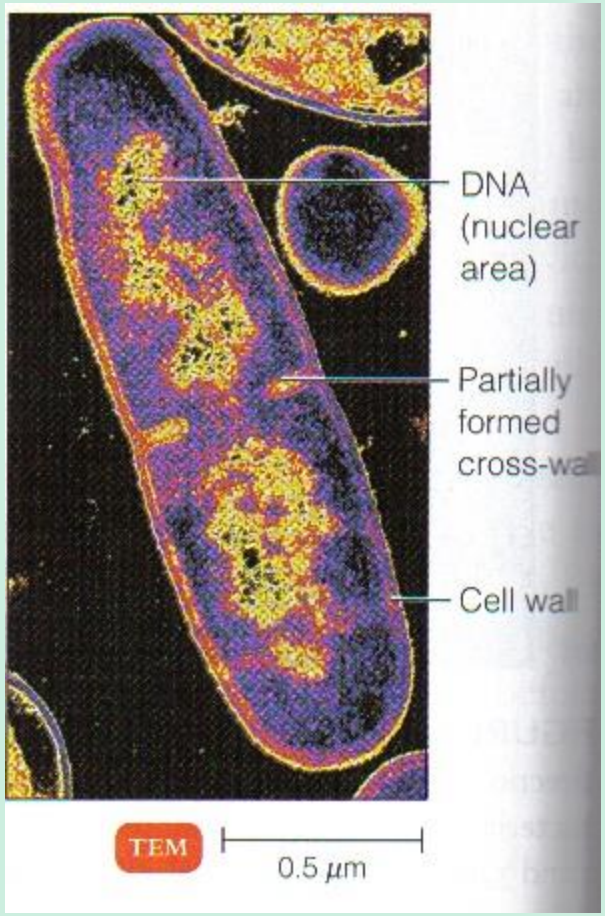
http://www.slideshare.net/Mona_Albureikan/presentation-5-41202526

Fig 6.11 Binary Fission

<u>Bacterial Growth</u> (= Multiplication)

1. Binary Fission





Generation Time

1. Time required for a cell or population to divide
   A. Less time is needed at optimum conditions
2. # organisms = $n \times 2^x$
   A. n = original number of organisms
   B. x = number of generations and/or doublings
3. Examples:
   A. If there is 1 cell to begin with, how many are there after 2 generations?
   B. After 6 doublings?
   C. If there are 4 cells to begin with, how many are there after 3 generations?

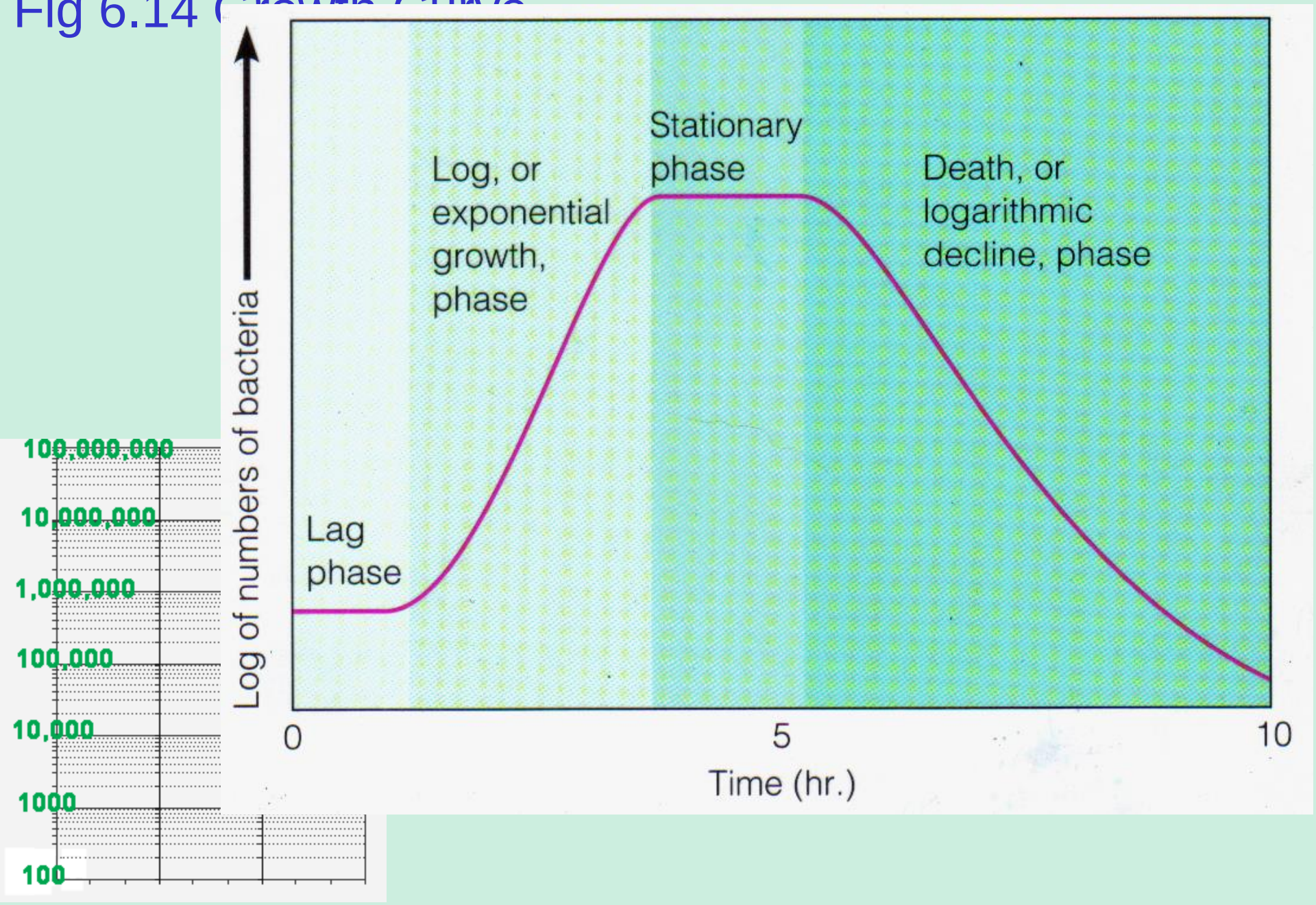
Fig 6.12 Arithmetic Numbers of Cell Division

Arithmetic Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

- If 1 bacteria reproduced every 20min, how many in 2 hours?
  - $1 \times 2^6 = 64$
- Trivia: *E.coli* w/20min generation time; 20 generations in 7 hours; 2^{20} = >1 million
- *E. coli* in 25.5 hours mass COULD = 80,000 ton aircraft carrier if didn't "death phase"
- Urine culture at room temp problems - refrig
- Video: The Multiplication Song by Simmonds Brothers

Generation Number	Arithmetic Number of Cells	Log_{10} of Arithmetic Number of Cells
0	1	0
5 (2^5) =	32	1.51
10 (2^{10}) =	1,024	3.01
15 (2^{15}) =	32,768	4.52
16 (2^{16}) =	65,536	4.82
17 (2^{17}) =	131,072	5.12
18 (2^{18}) =	262,144	5.42
19 (2^{19}) =	524,288	5.72
20 (2^{20}) =	1,048,576	6.02

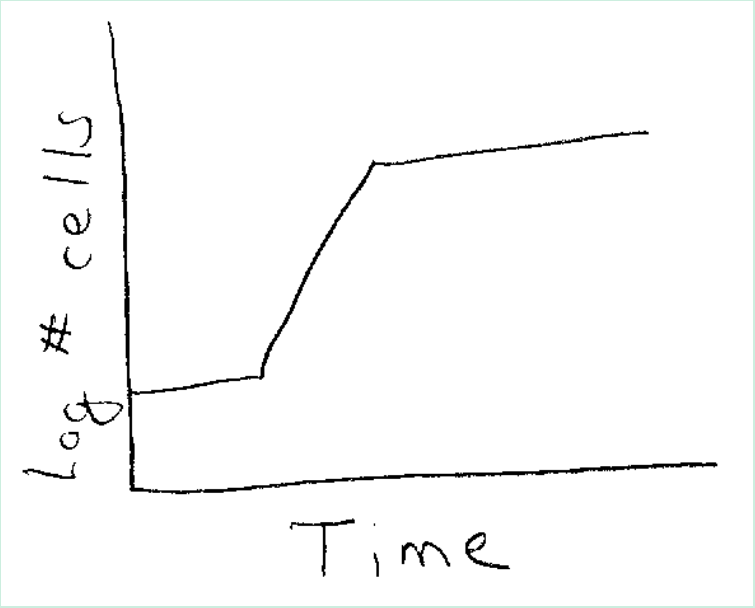
Fig 6.14 Growth Curve



Growth Curve

1. **Lag Phase** (Similar to interphase in mitosis)
   A. 1st placed in new medium: little (no) division
   B. Takes time to copy DNA & for enzyme synthesis
2. **Log Growth Phase**
   A. Exponential growth (logarithmic)
   B. Active metabolically
   C. Most sensitive to radiation, antibiotics, etc
3. **Stationary Phase**
   A. # deaths = # new cells
   B. ⇓ nutrients, ⇑ wastes, pH change
4. **Log Death Phase**
   A. Logarithmic decline, # deaths > # new cells

Example Graph Problems #1

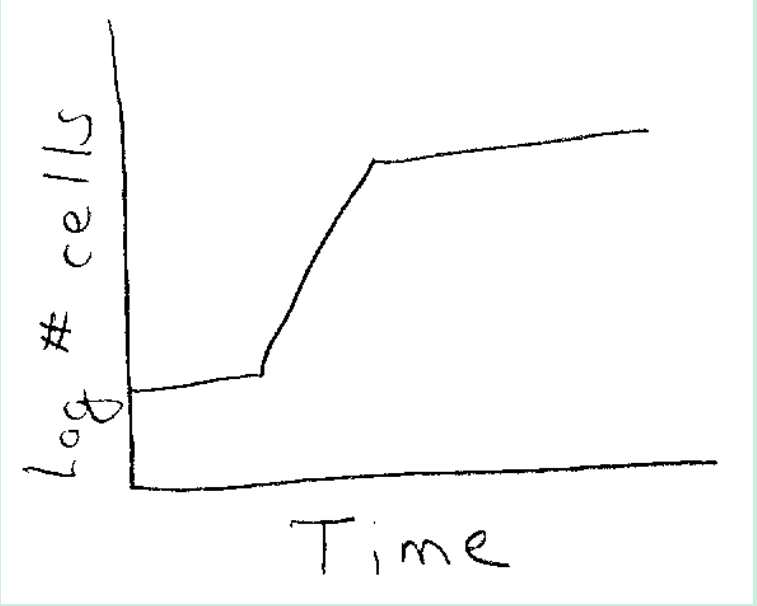


Graph shows growth at 25C

1. If organism has optimum temp of 20C, how would the graph change if:
   A. Grown at 30C instead?
   B. Grown at 20C instead?
2. If the organism is a psychrotroph, how would it change if:
   A. Grown at 5C?

Example Graph Problems #2

Need to do this example before assign end-of-chapter questions



Graph is on an agar which contains lactose.

1. If the organism can utilize lactose & peptones, similarly to *E. coli*, how would the graph change

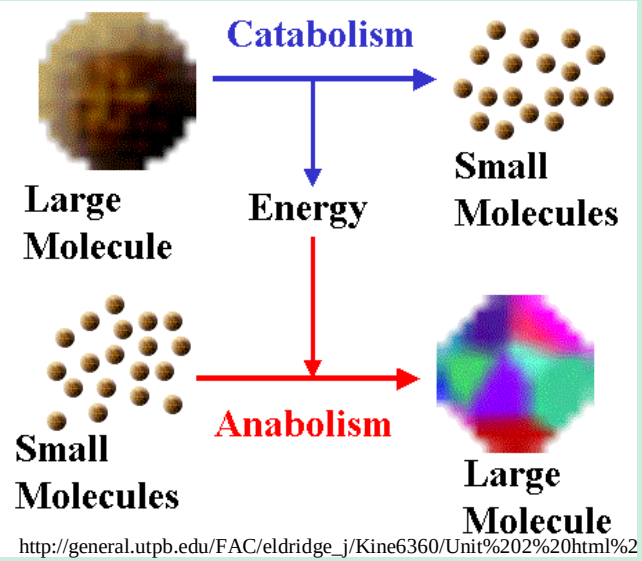
   A. If peptones are added?

   B. Lactose is doubled?

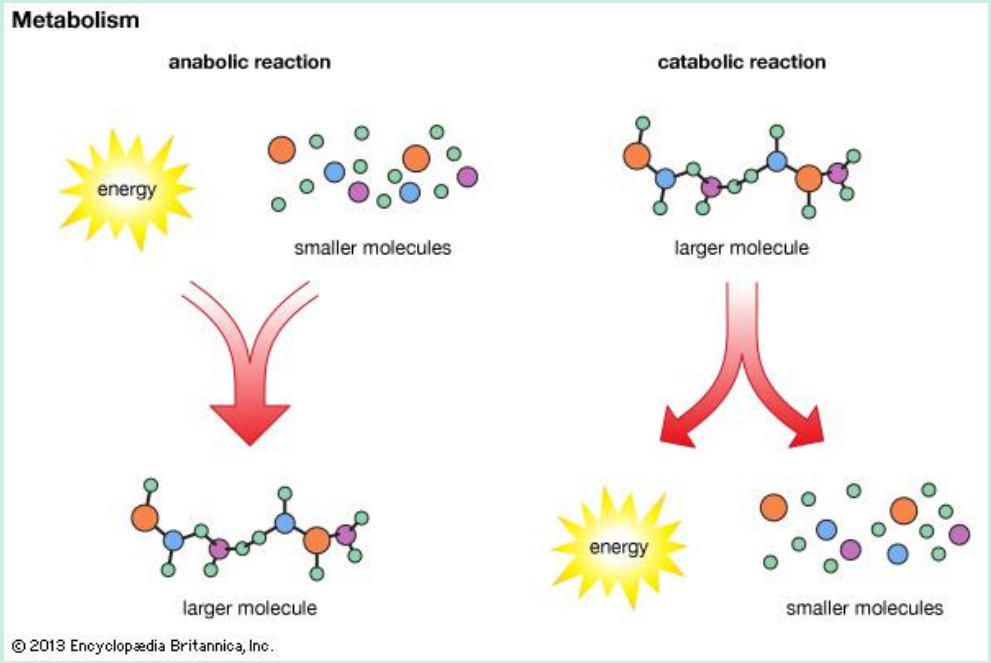
Lab #13 Carbohydrate Utilization

Anabolism vs. Catabolism:

All tests on Practical Exam –require explanation of YOUR results



http://general.utpb.edu/FAC/eldridge_j/Kine6360/Unit%202%20html%20file/Content%20Display%20%20Unit%202%20-%20Energy%20Metabolism_files/13G-catabolism&anabolism2.gif



http://media-2.web.britannica.com/eb-media/59/166059-004-40ACDC27.jpg

Anabolism

TERMS NOTE: All tests on Practical Exam –require explanation of YOUR results

1. Anabolism- "A" = "Adding" together
   - A. Example: Dehydration synthesis
     - i. Monosaccharides combine to form disaccharides (sucrose) & starch
     - ii. Dehydration accomplished removing OH- & H+ from diff molecules, linking the molecules where the OH- & H+ were.
     - iii. Energy is stored in the new bonds (ie – starch & glycogen store energy)
     - iv. Example equation: A + B → AB

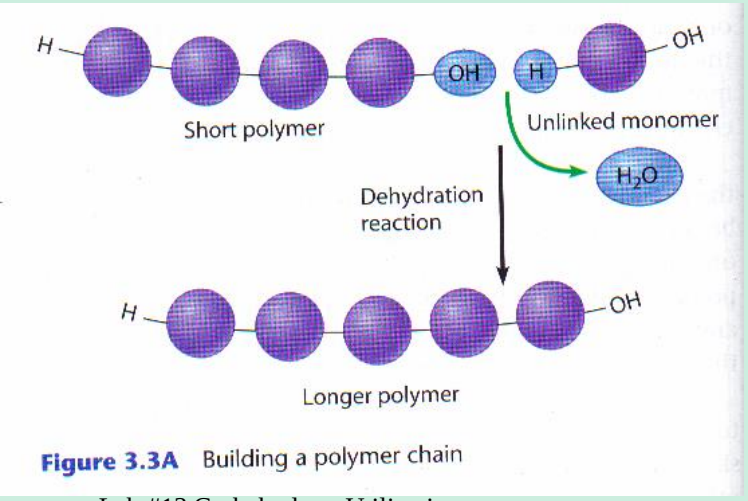
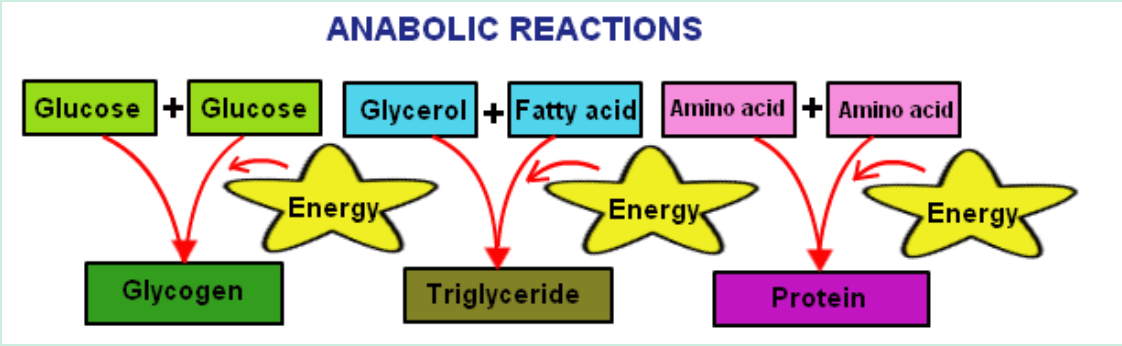


Figure 3.3A Building a polymer chain



http://images.tutorvista.com/cms/images/44/anabolic-reaction.png

Catabolism

2. Catabolism: "C" = "Cannibalism"

   A. "Breaking down" or Decomposition/hydrolysis

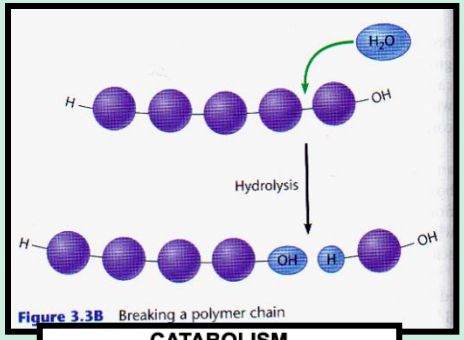
      AB → A + B

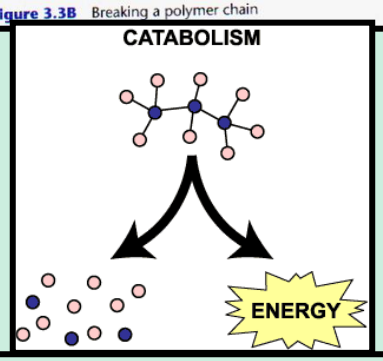
   B. Energy released when bonds are broken.

   C. Examples:

      i. "Burn" glucose for energy breaking it down into CO2

      ii. ATP broken into ADP + PO4 , releasing energy at cellular level





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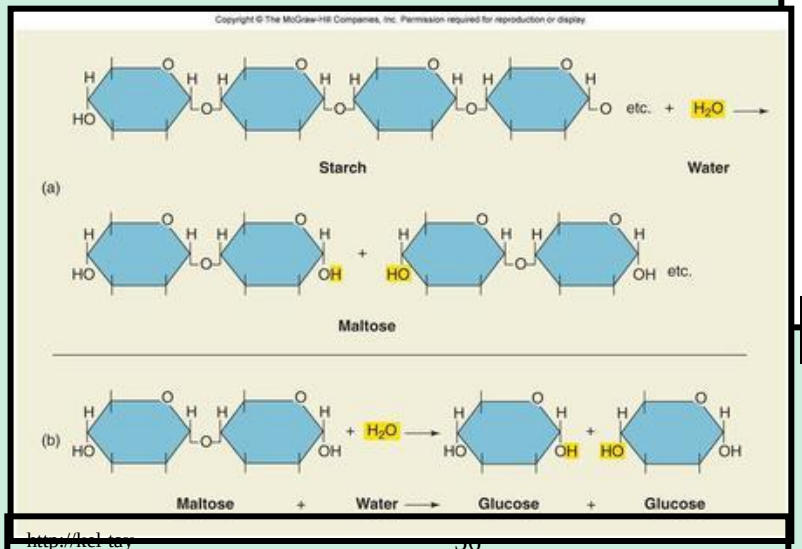

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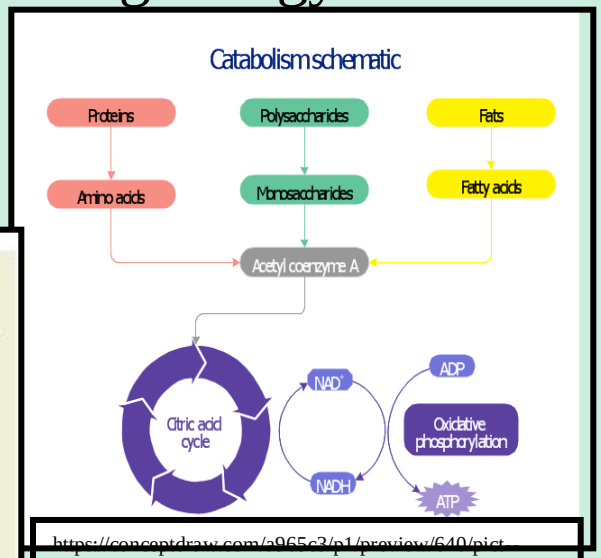
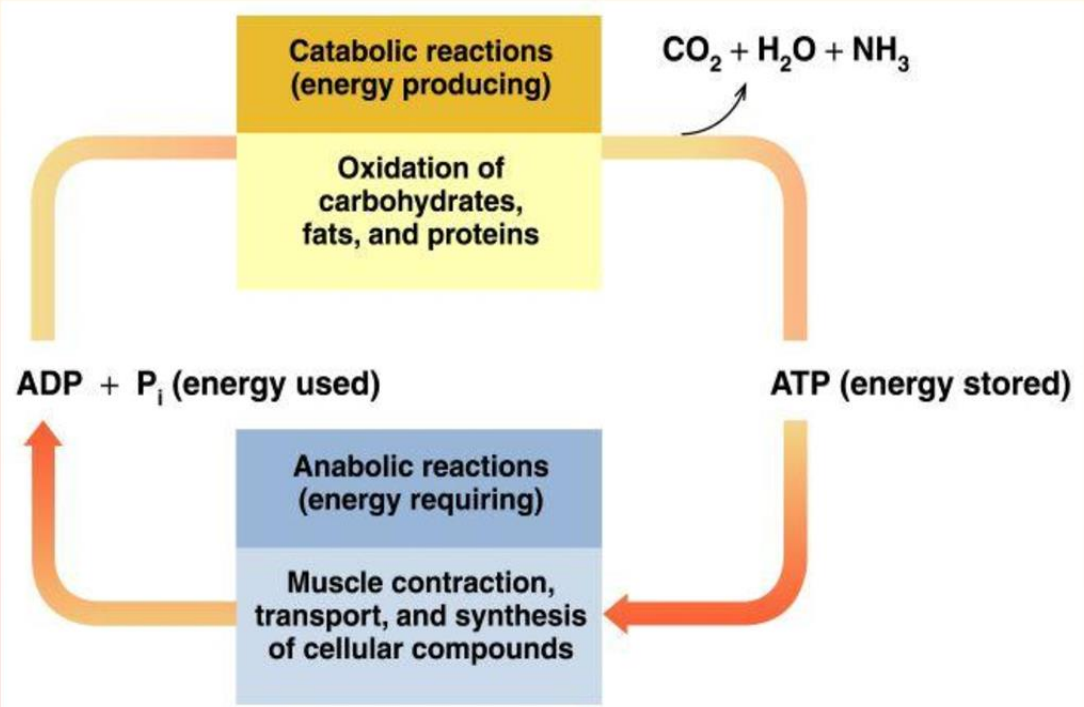


https://conceptdraw.com/a965c3/p1/preview/640/pict--catabolic-pathway-map-catabolism-schematic-biochemical-diagram

Metabolism Summary

Metabolism

- **Metabolism** involves two main processes, **catabolism** and **anabolism**
- **Catabolic reactions** break down large, complex molecules to provide smaller molecules and energy (ATP)
- **Anabolic reactions** use ATP energy to build larger molecules from smaller building blocks



http://img.docstoccdn.com/thumb/orig/518850.png

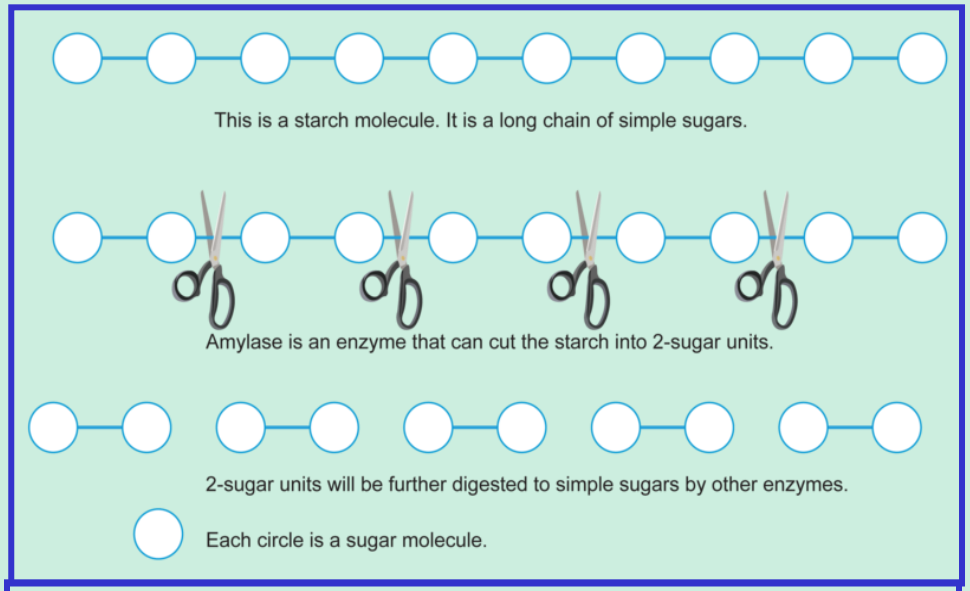
3. Exoenzyme vs. endoenzyme

Exoenzymes, Amylase

A. Exo: Enzyme is secreted & it's site of action is OUTSIDE the bacteria

   i. Amylase is an exoenzyme

   - Breaks down starch
   - Starch is too big to pass through cell membrane
   - Amylase begins the break down into glucose which is small enough to pass membrane



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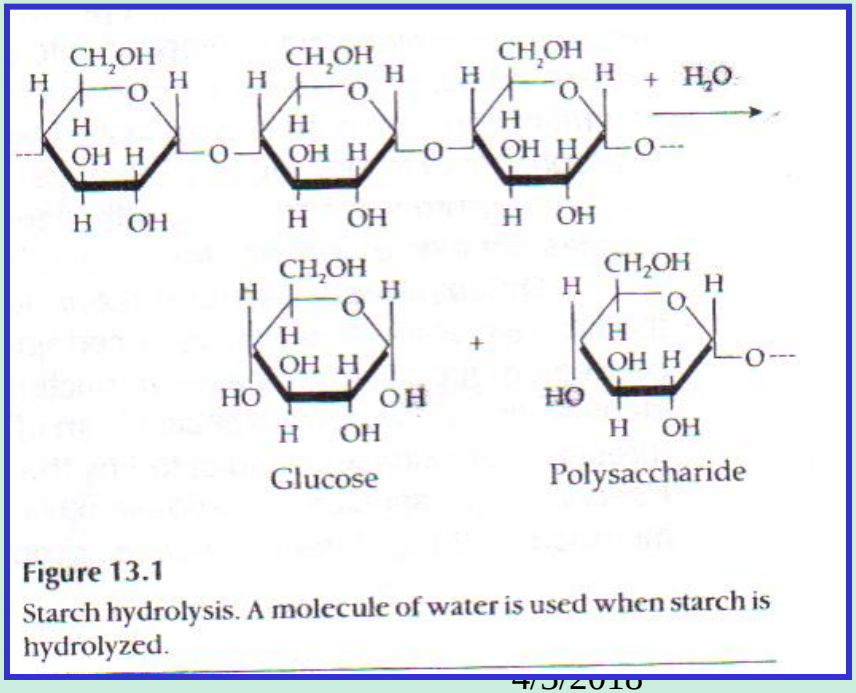


Figure 13.1
Starch hydrolysis. A molecule of water is used when starch is hydrolyzed.

Endoenzymes; Photolyases & Excision Repair Enzymes

B. Endo: Enzyme remains inside cell - it's site of action is INSIDE the bacteria

   i. DNA ligase: ties nucleotides together to form DNA chains

   ii. Photolyases & excision repair enzymes "repair" DNA after UV damage

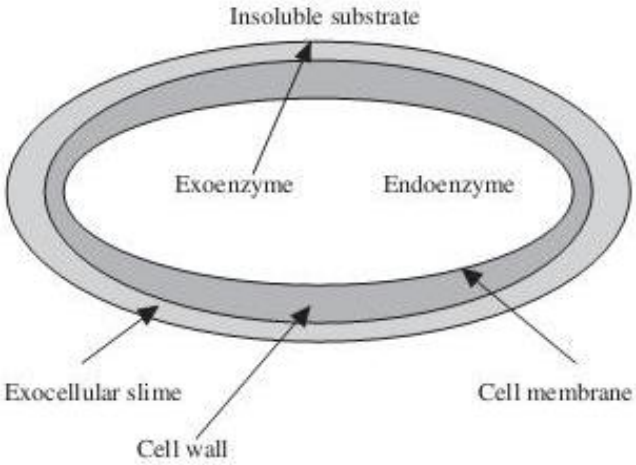


Figure 2.1 *There are two types of enzymes that are used by bacteria to degrade substrate. Exoenzymes are produced in the cell and released through the cell membrane and cell wall to hydrolyze insoluble substrate that is adsorbed to the exocellular slime. Soluble wastes enter the bacterial cell and are degraded by endoenzymes.*

http://1.bp.blogspot.com/-UbpV1YTtxrc/T0YS3cPWHcI/AAAAAAAABRc/2LlGJ0rj6ak/s1600/methane+baceria+5.JPG

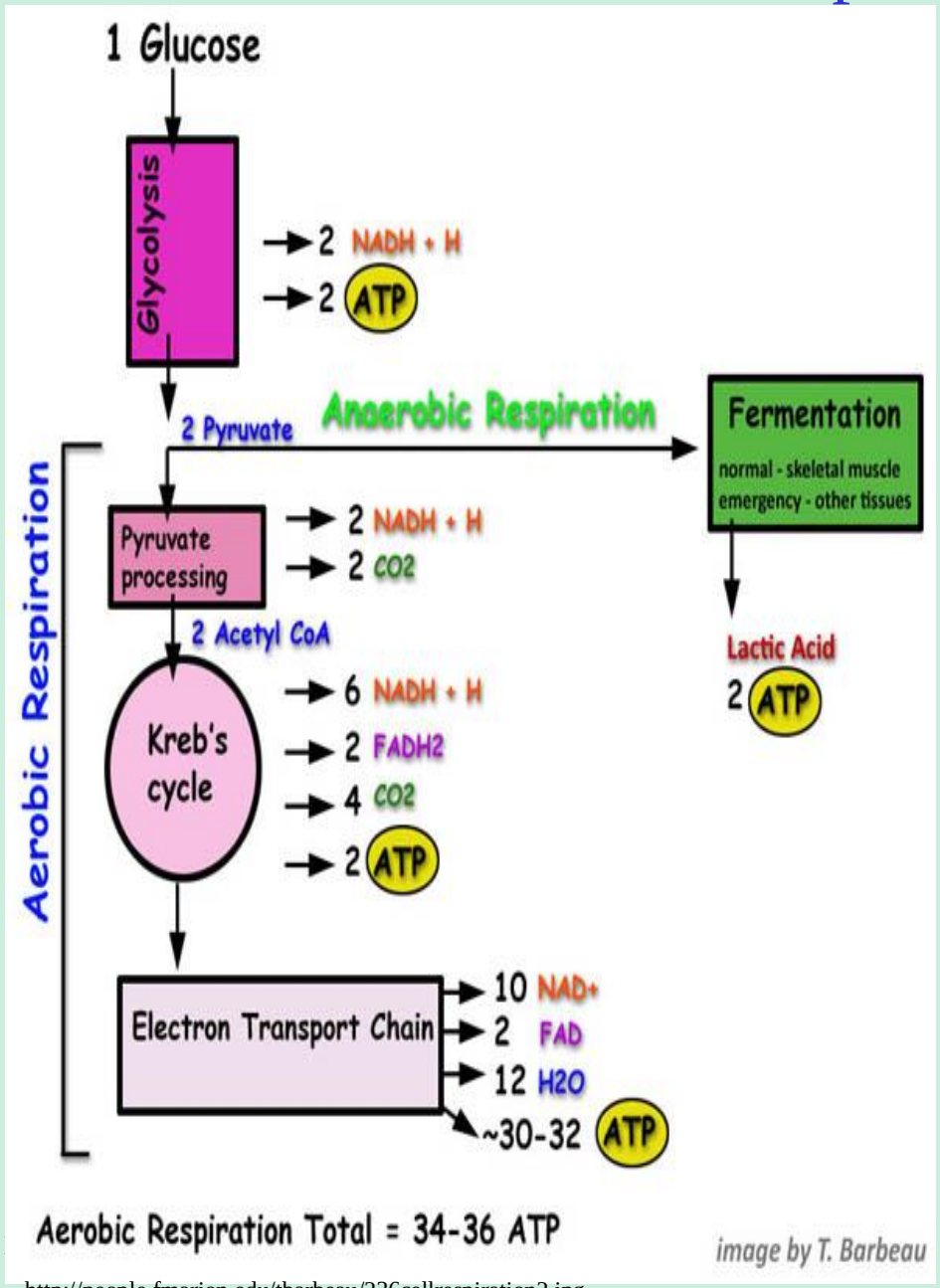
Growth, Metabolism, Endo/Exoenzymes

Exoenzyme:
https://www.youtube.com/watch?v=5ktLSmAm4ok

Metabolism/Catabolism:
https://www.youtube.com/watch?v=r-JuSnXoLHY

Growth:
https://www.youtube.com/watch?v=cZsVi3CaZ7s

Aerobic vs. Anaerobic Respiration Diagram



http://people.fmarion.edu/tbarbeau/236cellrespiration2.jpg

Examine the diagram

1.How many total ATP's are produced during Aerobic Respiration?

2.How many total ATP during Anaerobic Respiration? Why?

3.Facultative Anaerobes can grow both aerobically & anaerobically. Predict their growth (reproduction) rate anaerobically vs. aerobically based on the diagram.

4.Obligate Anaerobes – Predict their growth (reproduction) rate anaerobically vs. aerobically based on the diagram.

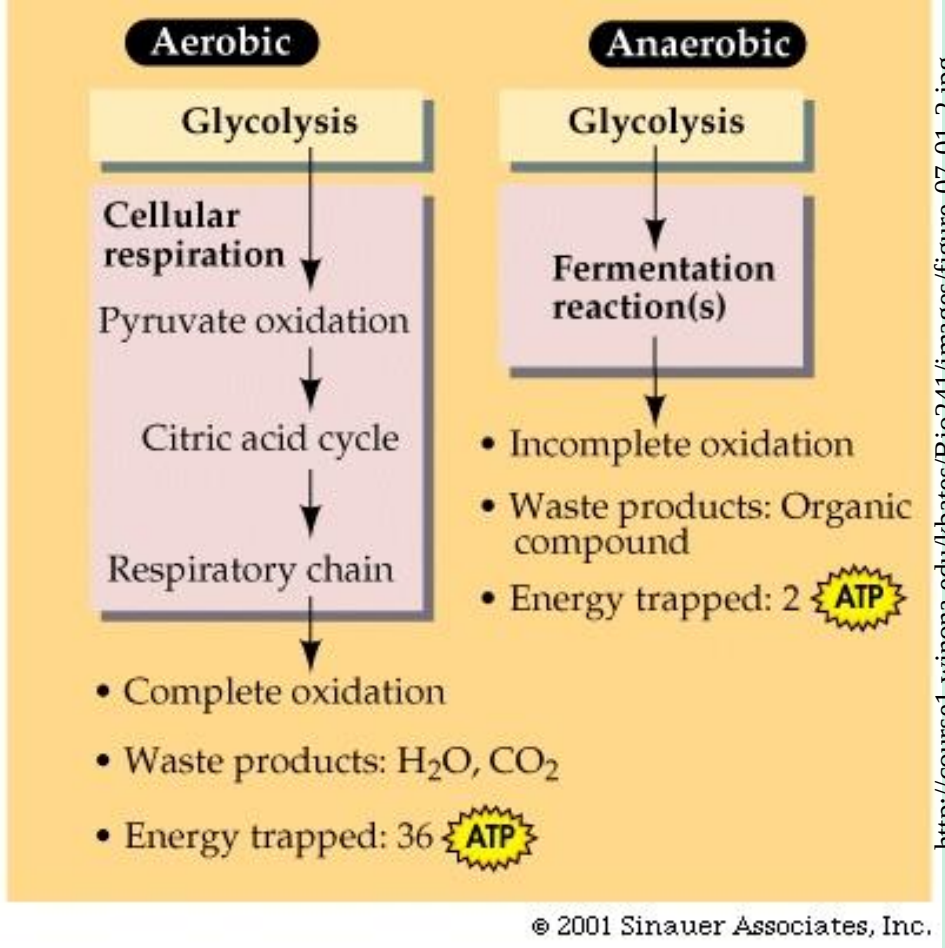
5.Which type of respiration involves fermentation? When our muscles undergo anaerobic respiration, what builds up?

Oxidative

4. Oxidative Organisms: REQUIRE molecular/atmospheric O2.

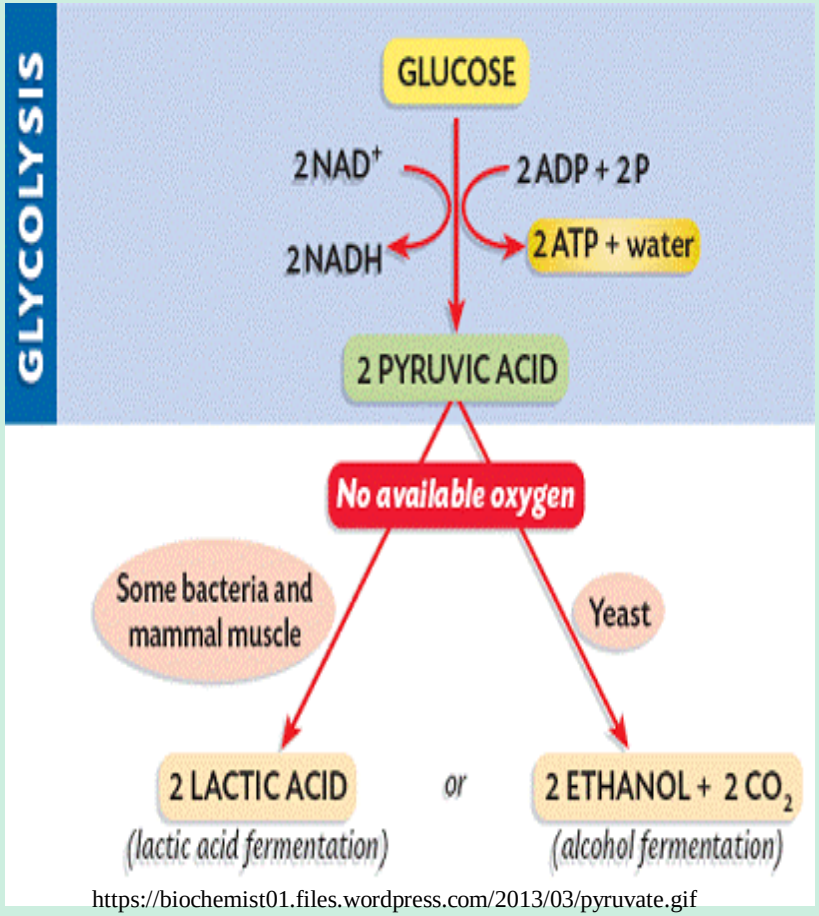
   A. OBLIGATE Aerobes who can ONLY do aerobic respiration.

   B. Can only break down carbs, fats, proteins for energy in presence of O2.



http://course1.winona.edu/kbates/Bio241/images/figure-07-01-2.jpg

Fermentative



https://biochemist01.files.wordpress.com/2013/03/pyruvate.gif

5. Fermentative: Doesn't require O2, but may or may not prefer O2.
   - A. Can use anaerobic respiration.
   - B. May or may not prefer aerobic respiration.
   - C. Most bacteria are facultative anaerobes, so most bacteria are classified as fermenters
   - D. Fermentation (anaerobic respiration) does not produce as much energy
     - i. By-products are alcohols & acids rather than breaking glucose all the way to CO2. Therefore some energy has not been released from the bonds.

Starch, Glucose, Iodine - Demo

- Do demo – test tubes of starch vs. glucose. Add I2
- Discuss plate set up - just a "Z" to see around edges.
- AGAR color, NOT colony color. May be best to look from bottom or hold plate over BLACK counter to show clearing.

Positive test: starch —amylase→ glucose subunits

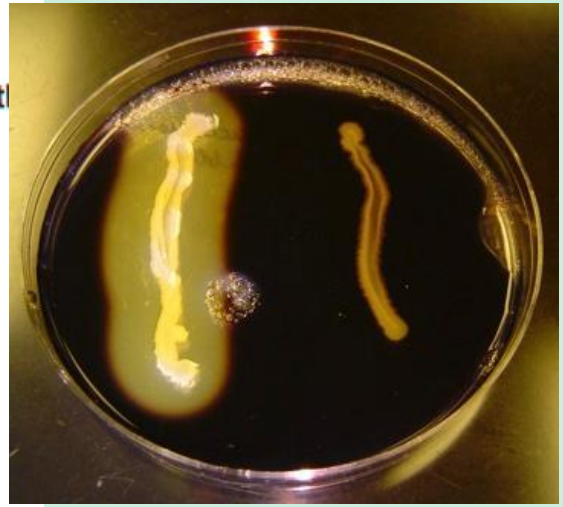
glucose subunits + added Gram's iodine = **clear zone around growth**

Example: ***Bacillus cereus***

Negative test: starch —no amylase→ starch

starch + added Gram's iodine = **purple-blue zone around growth**

Example: ***Escherichia coli***



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Starch Plate

Starch Plate:

1. Ingredients: Starch, beef extract, agar –Purpose of each?
2. Amylase hydrolyzes starch into? Starch + Amylase ⟶ Glucose subunits
3. Growth check – why must the organism have growth to interpret the test?
4. Results: Starch + Iodine = Change to deep brown color
   A. Clear **under/around** colony = **POS** for amylase (starch-hydrolyzed)
   B. Brown **under/around** colony = **NEG** for amylase as starch still present
5. Is amylase an exo vs. an endo enzyme? Explain based on observations.

Positive test: starch —amylase→ glucose subunits

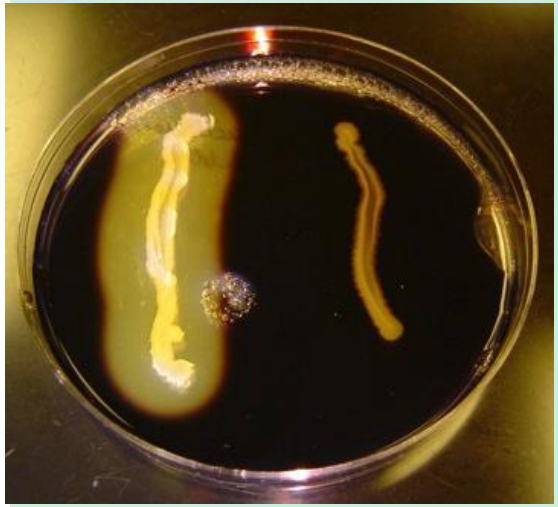
glucose subunits + added Gram's iodine = **clear zone around growth**

Example: ***Bacillus cereus***

Negative test: starch —no amylase→ starch

starch + added Gram's iodine = **purple-blue zone around growth**

Example: ***Escherichia coli***

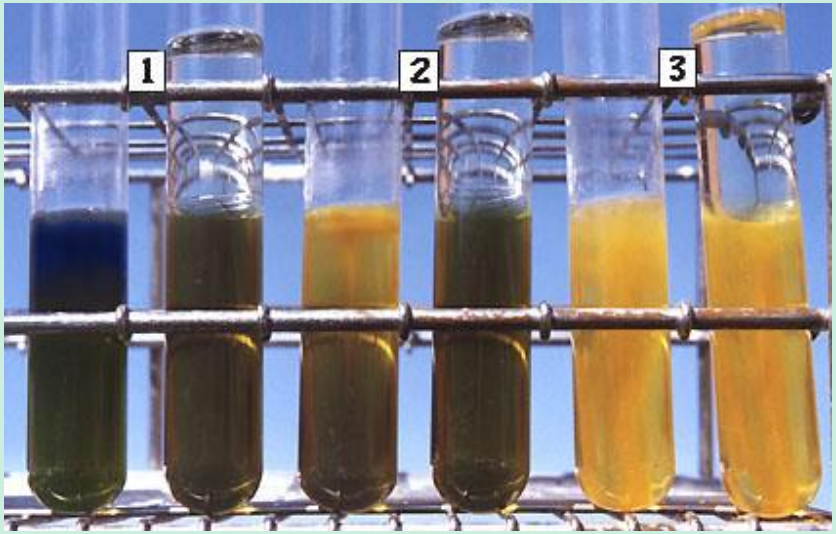


https://classconnection.s3.amazonaws.com/736/flashcards/817736/jpg/starch_hydrolysis1335001420652.jpg

OF-Glucose

1. Glucose, peptone, pH indicators, & low amount agar (semi-solid) Purpose of each?
2. Growth check Why must the organism have growth to interpret the test?
3. Results used to classify the organism into 1 of the following 3 terms:
   A. Oxidative
   B. Fermentative
   C. No glucose utilization. (So how does it grow? Reaction?)
   - NOTE: REQUIRES 2 tubes to classify – Why?

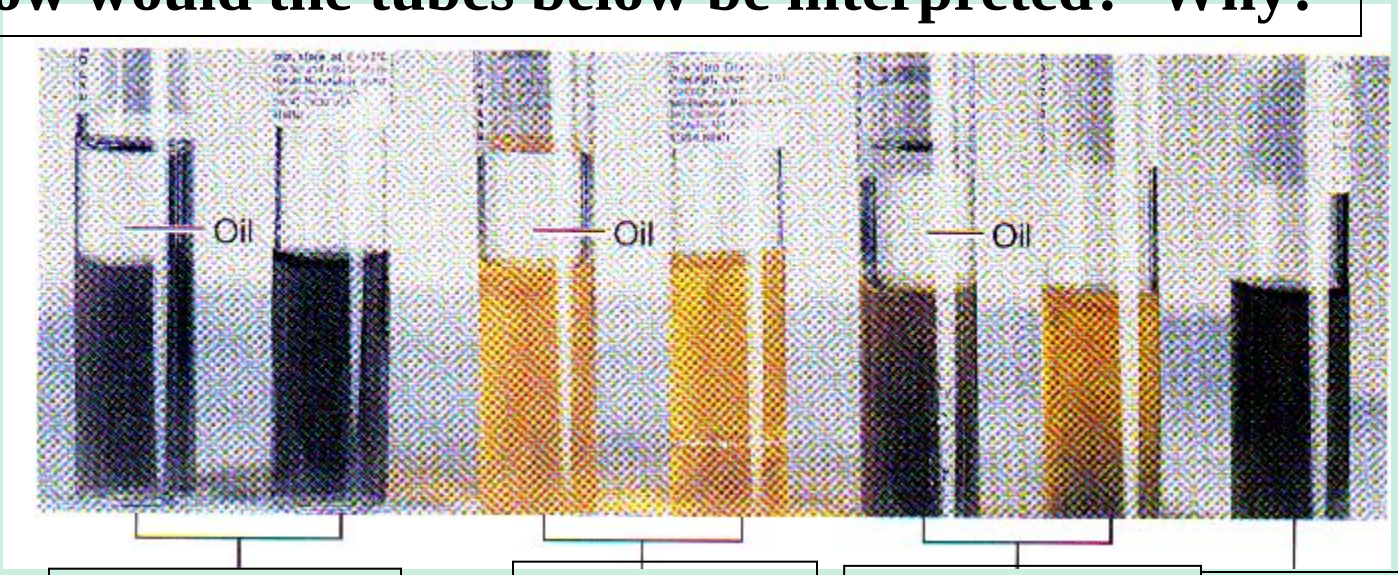
Demo set up – Refer to Lab #13 page 13-4. Needle straight down & back out so ONE line. Also check for growth.



http://www.jlindquist.net/generalmicro/DMimages/newglucof2.jpg

OF-Glucose Tube Examples-Avail in Atlas

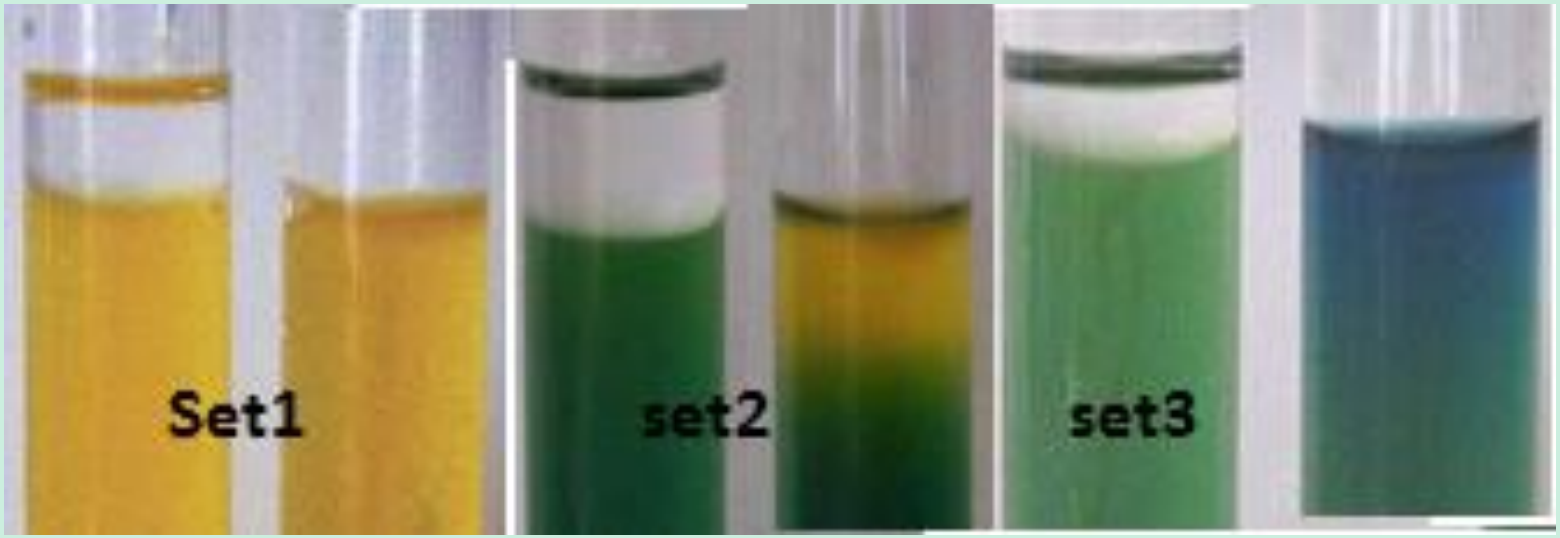
How would the tubes below be interpreted? Why?



-	F	O	Control
Non-utilizer	Fermenter Facultative anaerobe	Oxidizer Obligate Aerobe/ Microaerophile	Uninnoculated

OF- Glucose

- Classify the type of organism in each of the following sets of tubes
- Explain what causes the color in each tube



https://classconnection.s3.amazonaws.com/456/flashcards/709456/png/of_test1316912056976.png

OF-Glucose, continued

A. W/O oil: O2 present (no oil blocking air) -checks for oxidation

B. With oil: checks for fermentation

C. Results:

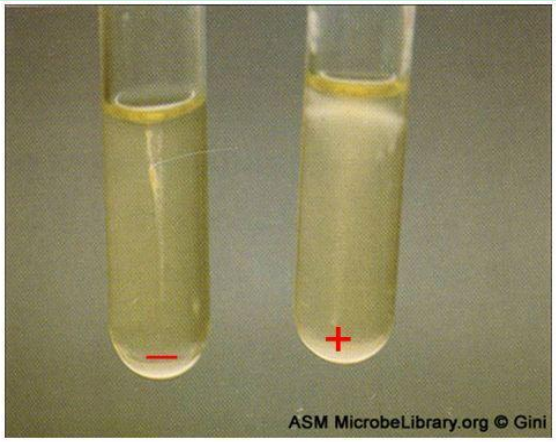
i. Both tubes color change to yellow: fermentative –Facultative Anaerobe.

ii. Tube w/o oil only turns yellow (top or all): oxidative – Obligate Aerobe

iii. No change/Blue-Green: Non-utilizer of glucose. Peptone used.

4. Motility (+ or -)

5. Gas



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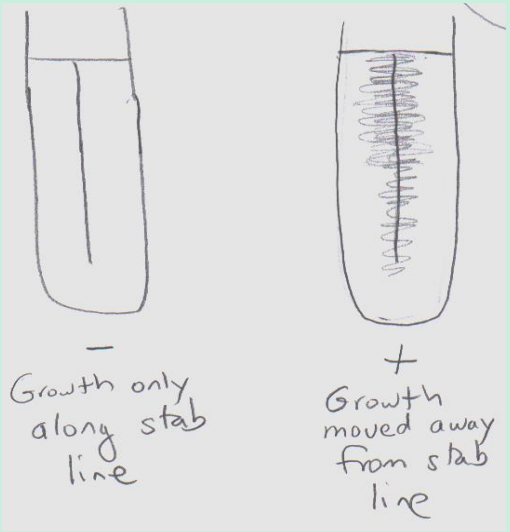


Flow Chart Available in Atlas

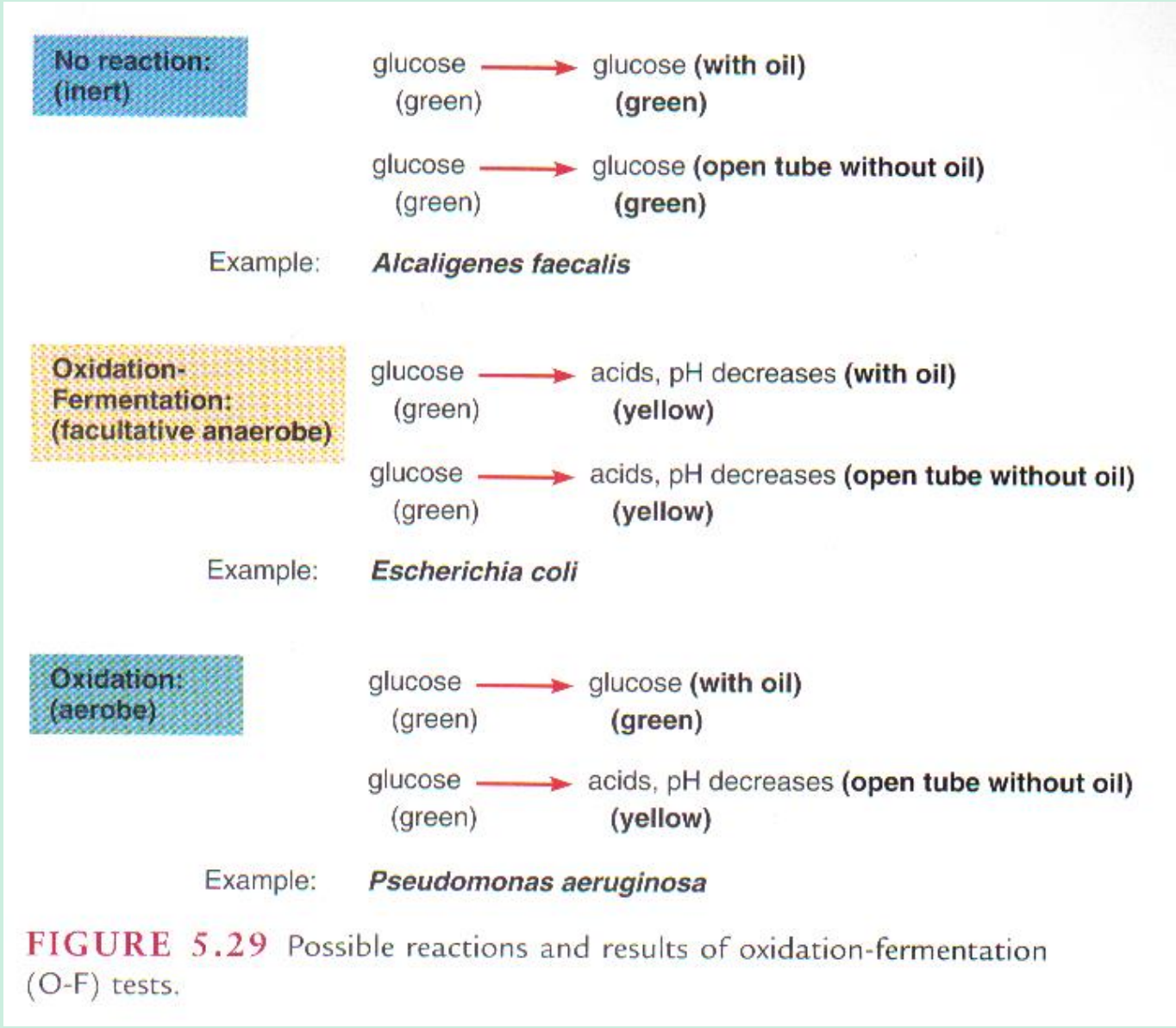


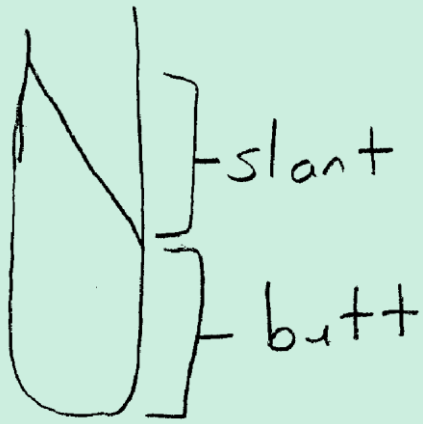
FIGURE 5.29 Possible reactions and results of oxidation-fermentation (O-F) tests.

TSI-Triple Sugar Iron

TSI Slants

1. Glucose, lactose, sucrose, peptone, Fe, indicators
2. Slant vs. butt

   Demo & discuss set up, see p.13-4
3. Results MUST be read at 24 hours.



BRIEF overview of visual changes:

A. Bubbles/Cracks: Gas

B. Black: Black H2S is produced when Sulfur is removed from cysteine (amino acid), and the Sulfur reacts w/Fe in slant

C. Carb utilization: Change from red/orange to yellow as acid is produced during carb catabolism

   i. NOTE: To determine color change MUST compare to an unused "control" tube)

   ii. See lab manual.

D. Record: Slant/Butt H2S +/- If gas present, circle butt symbol.

TSI Diagrams in Lab Procedure

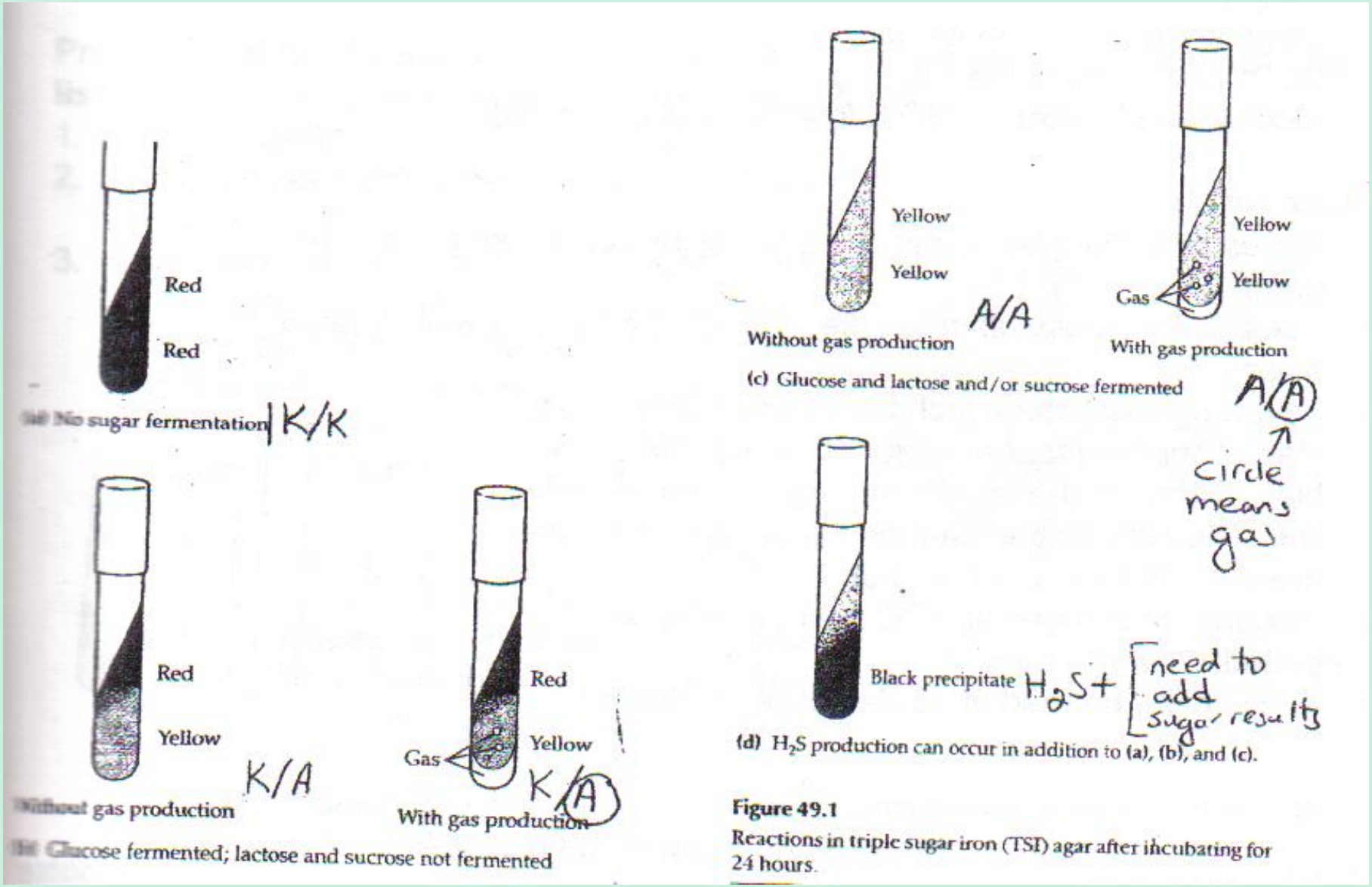


Figure 49.1
Reactions in triple sugar iron (TSI) agar after incubating for 24 hours.

TSI Mechanism – Glucose non-utilizer

1. Contains 1 part peptone, 1 part glucose, 10 parts lactose, 10 parts sucrose, Fe
2. Phenol red indicator: 7.4 red, acidic yellow, basic deeper red
3. **No sugar used:** Peptone oxidized only.
   - A. Peptones broken into??
   - B. Tube becomes even more alkaline & darker red
   - C. K/K OR Alk/Alk OR Red/Red (NOTE **Red = orig color or darker red)

Control
Uninnoculated

Deeper Red on SLANT (oxidizing)
compared to Control at 24 hours

TSI Mechanism – Glucose Only Used

4. Glucose is ONLY SUGAR used (peptone is also used)

A. Acid produced, entire tube yellow in 10 hours

B. BUT LITTLE Glucose present in tube, so it is used up quickly.

C. After glucose, peptones are oxidized. WHERE oxidized?

- SLANT reverts back to alkaline and is red again.
- BUTT stays yellow

D. K/A OR Alk/Acid or Red/Yell: (NOTE **Red = orig color or darker red)

Control **10 Hours** **24 Hours**

TSI Mechanism – Glucose AND Glucose/Sucrose/Both

5. Glucose & EITHER lactose or sucrose or both

A. 1st glucose used & tube turns yellow within 10 hours

B. THEN: 10x more lactose/sucrose, LOTS acid produced, whole tube stays yellow

C. A/A OR Acid/Acid OR Yell/Yell

Control

10 Hours

24 Hours

TSI Mechanism: H2S from Fe & Gas Production

5. ALSO RECORD

A. H2S **POS**: Sulfur removed from cysteine → H2S →reacts w/Fe & black butt formed

B. Gas produced? OBVIOUS bubble/crack. Circle butt symbol.

Interpret & Write the Symbols for the following TSI Tubes:





TSI Tubes – Interpret & Record Symbols

- Record appropriate symbols below each tube
- Later, come back & tell what sugars used & other reactions causing color

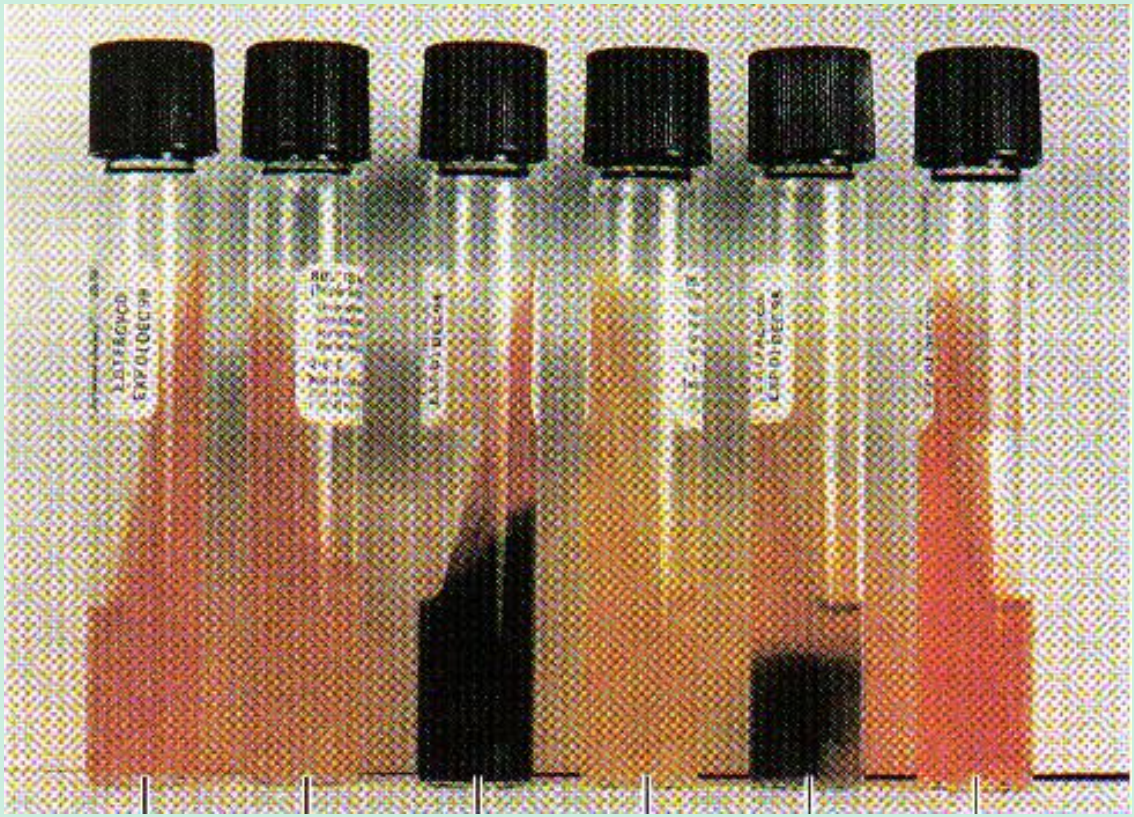
http://3.bp.blogspot.com/-hV8kGhovz20/UZnVcfNy3oI/AAAAAAAAAJ8/THSKBxdsgsA/s1600/New+Picture+(1).png

TSI Tubes – MORE tubes to Interpret, Symbols, Reactions

- Record appropriate symbols below each tube
- Which tube(s) clearly show peptones were utilized? Explain

TSI Tube Diagrams

How would the tubes below be interpreted? Why?



K/K H2S-	K/A H2S-	K/A H2S+	A/A H2S-	A/A H2S+	Control (Not innoc)

TSI Flow Chart Available in Atlas

No carbohydrate fermentation or hydrogen sulfide production:

glucose, lactose, sucrose (red slant/red butt) → glucose, lactose, sucrose **(red slant/red butt)**

cysteine → cysteine **(no black color)**

Example: ***Alcaligenes faecalis***

Glucose fermentation only:

lactose, sucrose (red slant) → lactose, sucrose **(red slant)**

glucose (red butt) → acids, pH decreases **(yellow butt)**

cysteine → cysteine **(no black color)**

Example: ***Shigella flexneri***

Glucose fermentation only with hydrogen sulfide production:

lactose, sucrose (red slant) → lactose, sucrose **(red slant)**

glucose (red butt) → acids, pH decreases **(yellow butt)**

cysteine → H_2S + other products

$H_2S + FeSO_4 \rightarrow$ FeS **(black color)**

Example: ***Salmonella typhimurium***

Lactose and/or sucrose and glucose fermentation:

lactose and/or sucrose (red slant) → acids, pH decreases **(yellow slant)**

glucose (red butt) → acids, pH decreases **(yellow butt)**

cysteine → cysteine **(no black color)**

Example: ***Escherichia coli***

Lactose and/or sucrose and glucose fermentation with hydrogen sulfide production:

lactose and/or sucrose (red slant) → acids, pH decreases **(yellow slant)**

glucose (red butt) → acids, pH decreases **(yellow butt)**

cysteine → H_2S + other products

$H_2S + FeSO_4 \rightarrow$ FeS **(black color)**

Examples: ***Proteus vulgaris*** ***Citrobacter freundii***

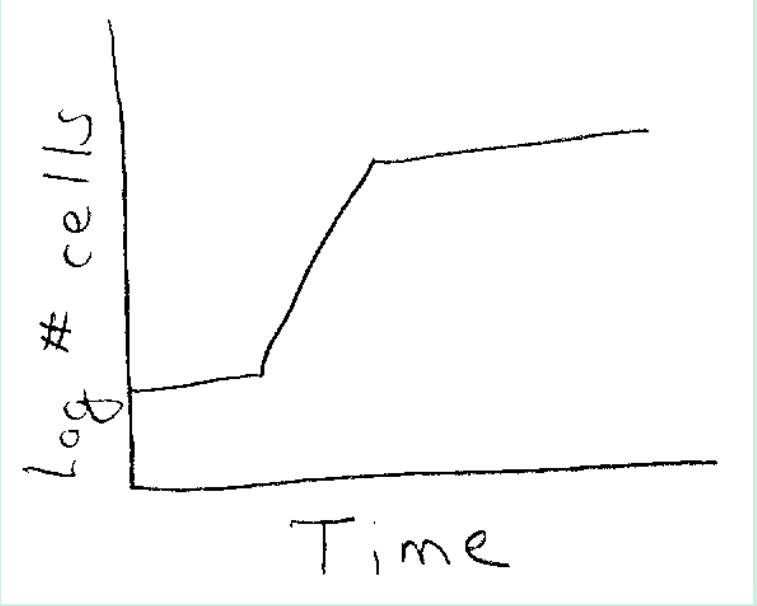
FIGURE 5.37 Possible reactions and results in triple sugar iron (TSI) agar and Kligler iron agar (KIA).

TSI Problems & Special Situations

1. Some bacteria can't utilize any of the sugars.
   A. How does TSI support their growth?
      i. Peptones used as nutrient
   B. What color does their tube turn? Why?

2. Organisms that utilize glucose will cause both the slant & butt to turn yellow, even if it can't use lactose & sucrose. If the slant is not read at 24 hours, the slant will turn back to red. Why?

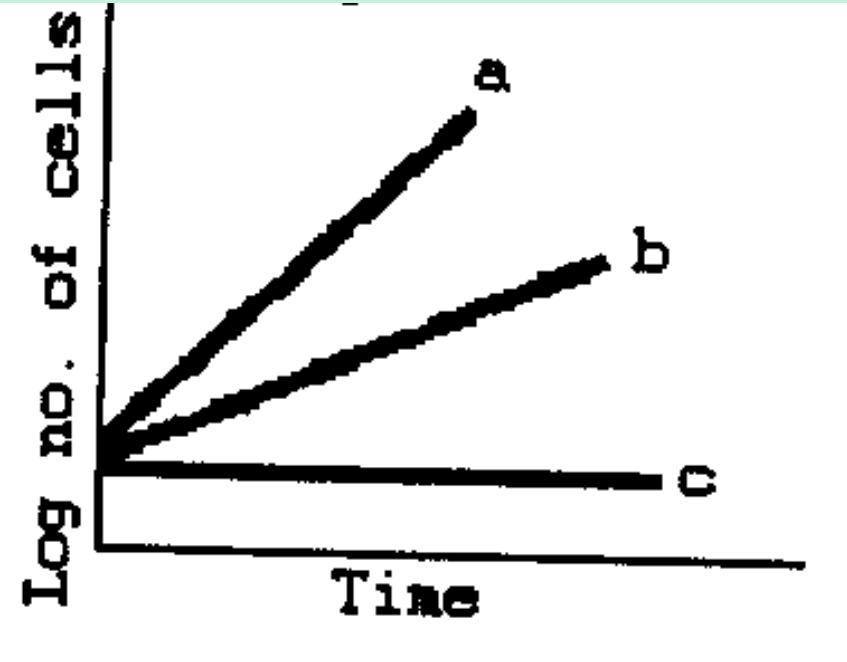
Example Graph Problems #3



Graph shows growth in room air. How would it change if it's a/an

1. Aerotolerant anaerobe grown in:
   A. Candle jar instead?
   B. Anaerobic conditions?
2. Facultative anaerobe grown in:
   A. Candle jar?
   B. Anaerobic conditions?

Growth Pattern Examples-Temperature Groupings



- Which line is a thermophile grown at 4C?
- Which line is a thermophile grown at 55C?
- Which line is a psychrotroph grown at 4C?

CHONPS – Used for??

	Carbs	Lipids	Protein	DNA/RNA	Special Notes
C					
H					
O					
N					
P					
S					

CHONPS – Used for??

	Carbs	Lipids	Protein	DNA/RNA	Special Notes
C	X	X	X	X	
H	X	X	X	X	
O	X	X	X	X	Respiration & energy production. Can be toxic
N			Amino Acids – NH_2	Nitrogen Bases – ladder "rungs"	
P		Cell Membrane (Phospholipids)		Phosphate Groups	ATP
S			Amino Acids (Cysteine)		

Review Miscellaneous #2: Selective vs. Differential Plates

Which of the following medias are:

1. Selective?
2. Differential?
3. General nutrient?

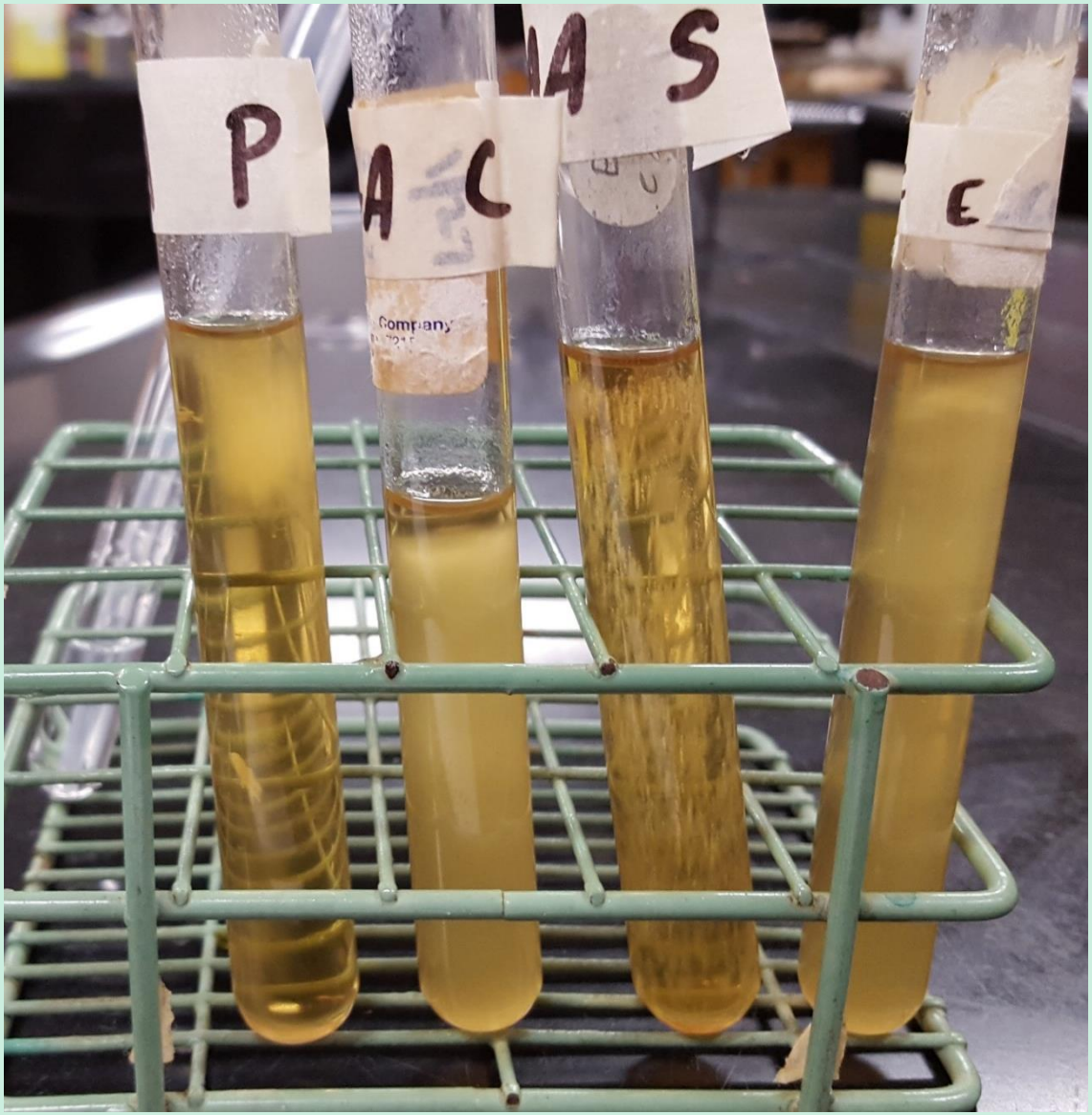
	Tryptose	Mac	MS	CNA
Staph epi	+	-	Pink	+
Serratia	+	Pink	-	-
Salmonella	+	Clear	-	-
Staph aureus	+	-	Yellow	+

Lab #19 Oxygen

Aerobic	Candle	Anaerobic	Classification	PREDICTED catalase	Organisms EXPECTED
+++	++/-	-	Obligate aerobe	+	Pseudo
-	-	++	Obligate anaerobe	-	Clostridium
+	+/++	++	Aerotolerant anaerobe	-	Enterococcus
+++	++	+	Facultative anaerobe	+	E. coli
++	+++	-	Microaerophile	+	None in this lab

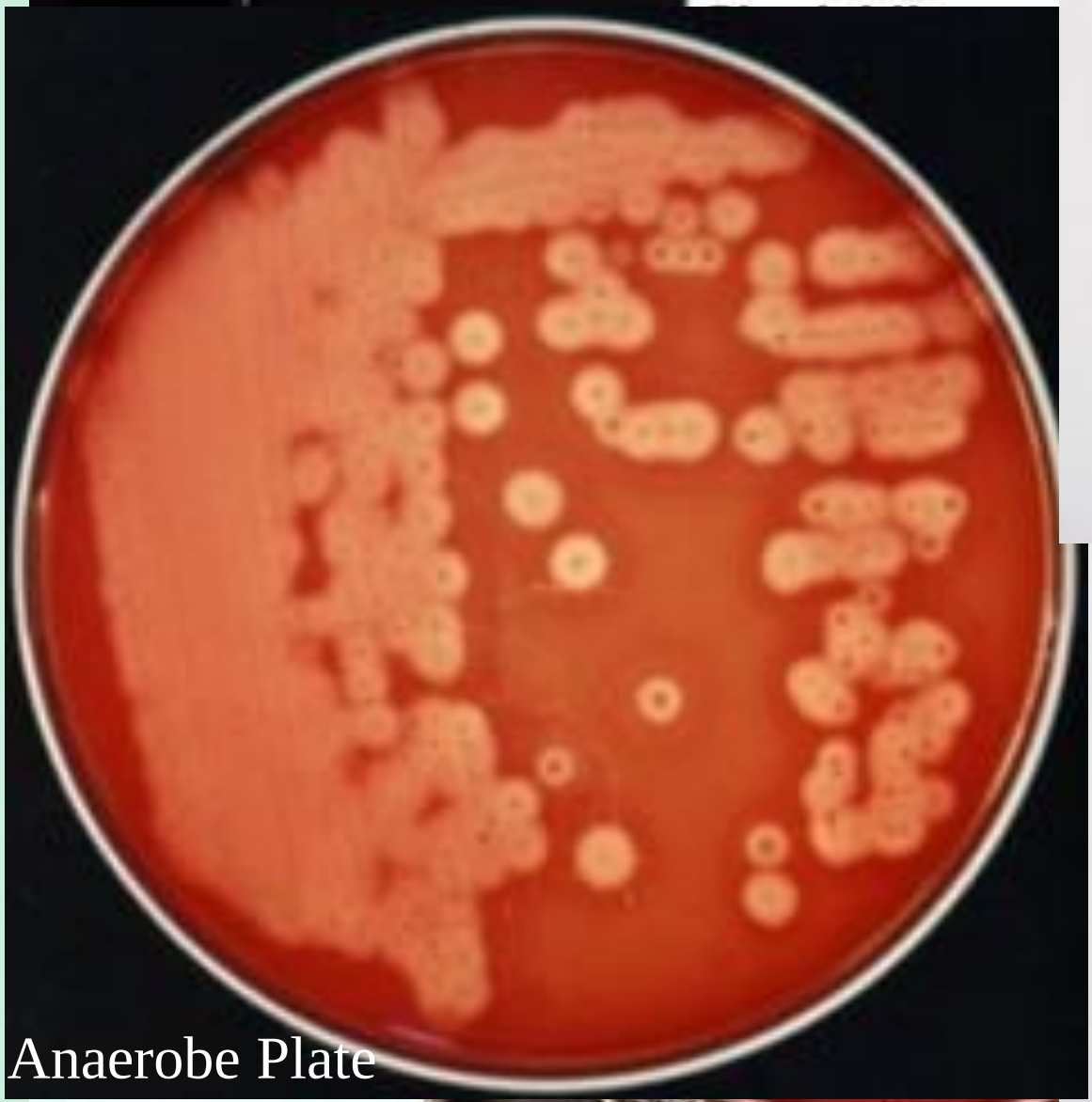
Lab #19 Oxygen Requirements – Plate Growth Expectations

Lab #19 Oxygen Requirements – Thioglycollate Broth Expectations



Pseudo ***Clos*** ***Strep*** ***E. coli***

Lab #19 Oxygen Requirements – Clostridium perfringens double hemolysis



Anaerobe Plate

Room Air – No growth

Candle Jar – No growth